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نموذج رقم (١٨)
اقرار والتزام بقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير

أنا الطالب: نافع خلف عيد إسماعيل الرقم الجامعي: ٨٠٧١٨٢١
التخصص: الوقاية النباتية الكلية: الزراعة

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير والدكتوراة عندما قمت شخصيا" باعداد رسالتي / اطروحتي بعنوان:
Impact of physical, chemical and microbiological properties of substrate on mycelial growth and yield of two strains of Agaricus bisporus.

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توقيع الطالب: التاريخ: ٢٤/ ٨ / ٢٠١١م



IMPACT OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SUBSTRATE ON MYCELIAL GROWTH AND YIELD OF TWO STRAINS OF *AGARICUS BISPORUS*

BY

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August 2011

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DEDICATION

بسم الله الرحمن الرحيم

" قل هل يستوي الذين يعلمون والذين لا يعلمون..."

My unlimited gratitude is devoted to my God "ALLAH", for complete help and success he awarded me.

To my mother for her support and praying.

To my brothers; Mahmood, Khaled and Aymen.

To my sisters; Hamda, Dalal, Kholoud and Muna.

To my wife Sahar.

To my lovely daughters; Shayma, Salsabeel, Raghad and Tasneem.

To my son Mohammed.

To all my dear friends and colleagues, who support and encourage me to reach my target.

AKNOWLEDGEMENT

My special gratitude and appreciation are due to my supervisor, Professor Ahmad R. Al-Momany for his kindness, patience and support during my study.

I am deeply indeed to the examination committee: Proff. Hifzi abu-Blan, Proff. Akel Mansour and Dr. Muwaffaq Karajeh, for their cooperation and comments.

My deepest thanks to my big brother agricultural engineer Fouad Hussain; the owner of the pioneer agricultural company for his help, advice and kindness.

I would like to thank Dr. Luma Albanna, for her help and encouragement.

I would like to thank Dr. Hosam Al titi, for allowing me to analyze samples in his lab.

I would like to thank Mr. Esmat Alkordi, Nabeel Abu Ruz, Rana Jado, Miss Kholoud Hamad, the secretary of the department of plant protection and Ashraf Hawamdeh for their great help and encouragement.

I would like sincerely to thank all my dear friends; Basel Khader, Riyadh Alzumor, Samer Habash, Amer Abu Shehab, Wafaa Khorfan, Dinah Hattab, Zayd Nabas, Ismael Ibrahim, Amjad Alyasory, Jaber Alyasory, Ghina Alhmood, shereen Abu- Zahra, Walid Weshah, Zakariya Al-Sayyed, Ahmed Suwad, Afrah Hammad, Mohhamad Ayyob.

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ABBREVIATIONS

AOAC: Association of Official Analytical Chemists.

BD : bulk density.

PD : particle density.

OM : organic matter.

MC : moisture content.

DM : dry matter.

TOC : total organic carbon.

EC : electrical conductivity.

WHC: water-holding capacity.

BE : biological efficiency.

CFU : colony forming unit.

Ppm. : parts per million.

F W M: fresh weight of mushroom.

F W C: fresh weight of compost.

DWC: dry weight of compost.

S M C: spent mushroom compost.

S D W: sterile distilled water.

IMPACT OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SUBSTRATE ON MYCELIAL GROWTH AND YIELD OF TWO STRAINS OF *AGARICUS BISPORUS.*

BY

NAFI' KHALAF AL MASAED

Supervised by

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Abstract

All experiments were conducted at the commercial mushroom farm (Pioneer Agricultural Projects Company, located in Almanarah / Aljiza 35 km south of Amman), during June 2010 to July 2011.

Compost samples from different stages of mushroom production in six growing rooms were analyzed for physical, chemical and microbiological properties, and the influence of these properties on yield and mycelial growth was evaluated. The number, size and weight of these fruiting bodies were affected by physical, chemical and microbiological properties of the substrate (compost). Compost pH ranged between 6.5 and 8.1, and it was decreased with time, and it was an indicator for incorrect preparation of compost when it was high. Bulk density (BD) was ranged between 0.58 and 0.78, and it was increased with time. In contrast, porosity was ranged between 87% and 96%, and it was decreased with time. Both bulk density and porosity were with no effects on yield of mushroom. Moisture content was ranged between 50% and 72%, it was decreased with time, the highest moisture was in phase I composting in room number 4 which produce the lowest yield (2465.6 kg) with exception of room number 6 which did not produce any yield. In contrast, dry matter ranged between 28% and 50%, and it was positively related to yield. Organic matter percent was ranged between 50% and 69% and there were no effects of organic matter on yield of mushroom, while ash content was ranged between 31% and 50% and it was also with no effect on yield of mushroom. Carbon to nitrogen (C/N) ratio was ranged between 10 and 22 and it was decreased with time during the crop cycle, but it was with no direct effect on yield of mushroom. Ammonia concentration was the most limiting factor which affected the quality of the compost; it was ranged between 0.0216 % and 0.1998 %. In rooms 4, 5, 2 and 1, the ammonia concentrations were 0.09816, 0.0861, 0.02558 and 0.0225 %, respectively, and the yields & productivities were 2465.6 kg & 12.3, 4400 kg & 22, 5140.7 kg & 25.7 and 5533.8 kg & 27.7, respectively. Certain fungi were dangerous and caused significant reduction (may reach 100%) of the yield, especially when they appeared early in the production season. *Rhizoctonia solani* was the most serious and aggressive fungus which competed with and / or parasitized on mushroom mycelium and caused a complete loss of mushroom production. It appeared in room number 6 after spawning, and caused apportion of the primordial formation, thus no pin-heads appeared and no

fruits were produced. Room number six produced no yield as a result of fungal disease (*Rhizoctonia* sp.) which appeared early after spawning, and prevented primordial formation. Room number 4 produced the lowest yield (2465.6 kg) as a result of fungal disease (*Fusarium moniliforme*) which appeared during the third flush and caused significant reduction of the yield (about 50%). White strains of *A. bisporus* (known as white button mushroom), were less productive, but more popular and there was great demand of them in the market comparing with brown strains (which known as Portobello), also white strains were more susceptible to diseases and undesirable conditions such as temperature and relative humidity. Mushroom fruits were ranged in dry matter % between 8.06 and 9.6, and there were no significant differences between flushes and also there were no significant differences between caps and stems of mushroom in dry matter percent.

1. INTRODUCTION

The increase in human population results in an increasing demand for food. Mushrooms are among the most available healthy food ingredients, which are low in calories, high in vegetable proteins, chitin, essential amino acids, vitamins and minerals (Tajbakhsh, *et al.*, 2008). Since ancient times, mushrooms have been consumed by humans not only as a part of the normal diet but also as a delicacy because they have a highly desirable taste and aroma (Colak, *et al.*, 2007). Mushrooms are fleshy, sometimes tough, umbrella like sporophores (Alexopolos *et al.*, 1979). They differ from green plants because they lack chlorophyll, and therefore they are heterotrophic. Some of them are saprophytes; others are parasites, while some of them are facultative (Chang, 1999, Ananbeh, 2003). Mushrooms are the fruiting bodies of macro fungi. Mushroom fungi include edible; medicinal and poisonous species. Edible mushrooms once called the "food of the gods" and still treated as a garnish or delicacy can be taken regularly as part of the human diet (Chang, *et al.*, 2008). Fungi are ideal food because they have a high content of protein (typically 20 – 30% dry matter as crude protein) which contains all of the essential amino acids. Fungal biomass is also a source of dietary fiber, good vitamins and is virtually free of cholesterol (Moore and Chiu, 2001, Khraisat, *et al.*, 2010).

Furthermore, over 200 species have been collected in the Far East region from the wild mushrooms and used for various traditional medical purposes. The most cultivated mushroom worldwide is white button mushroom (*Agaricus bisporus*) (Sanchez, 2004, Dundar and Yildiz, 2009).

Mushroom cultivation technology is friendly to the environment (Chang, *et al.*, 2008). Therefore, the consumption of mushrooms in the world is growing every year. The highest consumption of mushrooms exists in Australia with 2.36 kg per capita in 2002

followed by Europe with 1.52. The lowest consumption exists in the Middle East and Africa with about 0.05 – 0.01 kg per capita (Sassine, *et al.*, 2005).

The commercial cultivation of edible mushrooms has expanded all over the world and production rates increased from 300,000 tones in 1970 to over 3 million tones of fresh weight mushrooms in 2003 (Diamantopoulou and Philippoussis (2001), Van Griensven, *et al.*, 2005).

The improvement and development of modern technologies, such as computerized control, automated mushroom harvesting, preparation of compost, production of mushrooms in a non-composted substrate, and improving methods of substrate sterilization and spawn preparation, will increase the productivity of mushroom culture. All these aspects are crucial for the production of mushrooms with better flavor, appearance, texture, nutritional qualities, and medicinal properties at low cost. Mushroom culture is a biotechnological process that recycles lignocellulosic wastes, since mushrooms are food for human consumption and the spent mushroom compost can be used in different ways (Sanchez, 2004, Fan*, et al.*, 1999). It might be the only current process that combines the production of protein-rich food with reduction of environmental pollution. Their nutritional value can be compared to those of eggs, milk, and meat. Mushrooms also contain vitamins and an abundance of essential amino acids (Andrade, *et al.*, 2008, Sanchez, 2010). The spent compost which left after harvesting the mushrooms can be used as a livestock feed supplement and as organic garden mulch, which is good for the soil (Najjab, 1995, Chang, *et al.*, 2008). *Agaricus bisporus* is produced from wheat straw, straw-bedded horse manure, chicken manure and gypsum. The casing layer is an essential part of the total substrate in the artificial culture of *A. bisporus*. Although many different materials may adequately function as a

casing layer, peat is commonly used and recommended as a good casing in mushroom cultivation (Colak, 2004).

Most mushrooms produce spores that are uninucleate and genetically haploid (1N). This means each spore contains one nucleus and has half the complement of chromosomes for the species. Not all mushroom species have basidium that produces four haploid spores. *Agaricus brunnescens* (*Agaricus bisporus*), the common button mushroom, has basidium with two diploid (2N) spores. This means each spore can evolve into a mycelium that is fully capable of producing mushrooms. *Agaricus bisporus* is one example of a diploid bipolar species (Stamets and Chilton, 1983).

An apparently older name for this mushroom is *A. brunnescens*, referring to the oxidative "browning" reaction when the mushroom is bruised. *Agaricus bisporus* has increased in popularity with the introduction of two brown strains, Portabella (sometimes also spelled Portobello, portabello, or portobella) and Crimini. The three mushrooms are all actually the same species. Portabella is a marketing name. The mushroom industry came up with more flavorful brown strains of *A. bisporus* that are allowed to open to expose the mature gills with brown spores; crimini is actually the same brown strain that is not allowed to open before it is harvested. The methods for growing *Agaricus* are vastly different from growing most other sorts of mushrooms. *Agaricus* is a secondary decomposer, which means that bacteria and other fungi have to break down raw materials before *Agaricus* can grow. This process known as composting. Other kinds of cultivated mushrooms such as oyster mushrooms and shiitake are primary decomposers of wood, and composting is not necessary (Volk and Ivors, 2001). In the process of composting, microorganisms break down organic matter and produce carbon dioxide, water, heat, and humus, the relatively stable organic end product. Under optimal conditions, composting proceeds through three phases; 1) the

mesophilic, or moderate – temperature phase, 2) the thermophilic, or high – temperature phase, and finally, 3) cooling or maturation phase (Trautmann and Olynciw, 2001).

The formulation of compost is crucial to carry on composting for *Agaricus* sp. and it is essential for an adequate balance between nutrients, especially carbon and nitrogen. As a result, mycelium growth can be influenced by a reduction or excess concentration of nutrients, accumulation of toxic products, production of secondary metabolites and changes in factors such as pH, which consequently can influence the mushroom production (Sharma, *et al.*, 2000, Andrade, *et al.*, 2008).

There are only five commercial mushroom farms in Jordan, and the owners are small growers who try to prepare their compost by themselves, but the little experience and the scarcity of the main ingredients of compost are the main problems in the way of success. Some growers prefer to import spawned compost from other countries, as Syria, Turkey and Holland. Because of drought in recent years, there were no sufficient quantities of wheat straw in Jordan, and the price is very expensive (about 160JD/ ton). Other problem in preparing compost locally is the low productivity in comparison with the imported compost (about 17 – 20 kg / m^2 compared with 25 – 30 kg / m^2).

The most important problems that face local mushroom related experimentation were scarcity of references about cultivation of mushroom in Arabic countries, scarcity of raw materials (e.g. wheat straw, horse – bedding manure and chicken manure) and the scarcity of market information about quantities of mushroom produced and consumed in Jordan.

The production of mushroom in Jordan is not sufficient and cannot cover the increased demand and the consumption requirements of people in Jordan, so the imported quantities of fresh mushroom increased from 155kg which costed 1,553 JD in 2004 to 871 kg which coasted 2,953 JD, while the imported quantities of canned mushroom

decreased from 1.6 million kg which costed about 790,000 JD in 2003 to 1.5 million kg which costed about one million JD in 2007 (Ministry of Agriculture, 2009).

Therefore the objectives of this work were:

1. To study the effect of physical properties of the substrate such as porosity, bulk density and organic matter on the mycelial growth and yield of brown and white strains of *Agaricus bisporus*.

2. To study the effect of chemical properties of the substrate such as moisture, ash, pH, and C: N ratio on the mycelial growth and yield of brown and white strains of *Agaricus bisporus*.

3. To determine the fungal microorganisms, that contaminate the substrate, which drastically affect the growth and yield of the mushroom.

4. To determine the most limiting factor of mushroom production in Jordan.

2. LITERATURE REVIEW

2.1 Morphology of White Button Mushroom

Although *A. bisporus* (Lange) Imbach has been cultivated since the 17th century in France and despite the commercial value of the button mushroom, the genetic improvement of this species has remained poor until the end of the 20th century. This species is perhaps the best known of all edible mushrooms (Rodier, *et al*., 2000). *Agaricus brunnescens* literally, the name means the fungus that becomes brown, probably referring to the color change of the flesh upon bruising. Also called *Agaricus bisporus* for the two spored basidia populating the gill faces. *Agaricus bisporus* a robust, thick fleshed *Agaricus* species, with thin gills that are pinkish when young, and darkening to dark brown and then chocolate brown in age. The convex cap is characteristically brownish, whitish or cream colored. The cap surface is smooth and dry, and between 5 – 10 cm in diameter. This species has a short, thick stem (3 – 6 cm long and 1 – 2 cm thick) which is adorned with a persistent membranous annulus from a well-developed partial veil. Its flesh stains very slightly red. Its spores are chocolate brown in mass (Gerrits, 1988 and Laessoe, 1998). *Agaricus bisporus*, like all fungi, are heterotrophic organisms, which means that for their nutrition and metabolism they depend on carbon sources formed earlier (during photosynthesis) by green plants. The carbon sources involved in the nutrition of the mushroom are present in straw and in animal manure (Gerrits, 1988).

2.2. Scientific classification of White Button Mushroom

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Sub-class: Holobasidiomycetidae

Order: Agaricales

Family: Agaricaceae

Genus: Agaricus

Species: *A. bisporus*. (Alexopoulos and Mims, 1995).

2.3 Species of White Button Mushroom

Around 90 species of *Agaricus* are found in Europe, and there are likely to be over 200 species world – wide, of these species, *A. bisporus* is the premier cultivated mushroom and grown throughout the world. The so-called "hot" mushroom, *A. bitorquis* (spring agarics) has been grown on a minor scale as an alternative to *A. bisporus* and two other species, the horse mushroom *A. arvensis* and *A. blazei* have been grown for their flavor (Calvo-Bado, *et al.*, 2000). Also there are other species of Agaricus; such as: *A. sylvicola* (woods agaricus), *A. Augustus* (prince agaricus), *A. campestris* (meadow agaricus), *A. bernardii* (salt- loving agaricus), *A. sylvaticus* (red- staining agaricus), *A. porphyrizon* (purplish yellow agaricus) and others, there are three poisonous species; *A. meleagris*, *A. xanthoderma* (toxic yellowing agaricus) (Laessoe, 1998, Tawayah, 2008), and *A. praeclaresquamosus,* toxic scaly agaricus (Laessoe, 1998).

Most commercial mushrooms are produced from one species; *Agaricus bisporus*. Within the species there are several distinct strains or lines. There are white, off-white, cream or brown strains; this indicates the color of the mushroom cap at maturity. Each

strain has its specific characteristics: cap shape, color, size and growing habitat (Rinker, 1986).

2.4. Nutritional value of *Agaricus bisporus* per 100 grams

Energy:	94 K J (22K cal.).
Carbohydrates:	3.28 g
Sugars:	1.65 g
Dietary fiber:	1.0 g
Fat:	0.34 g
Protein:	3.09 g
Water:	92.43 g
Thiamine (Vitamin B1):	0.081 mg.
Riboflavin (Vitamin B2):	0.402 mg.
Niacin (Vitamin B3):	3.607 mg.
Pantothenic acid (B5):	1.497 mg.
Vitamin C:	2.1 mg. USDA, 2010.

2.5. Compost for growing mushrooms:

The growth of mycelium on compost is faster than the growth on any other substrate, probably due to the available nutrients metabolized by certain microflora (Gerrits, 1988 and Tawayah, 2008). Specific compost is used for the production of mushrooms. It will be prepared in two phases (Gerrits, 1988, Mamiro, *et al*., 2007).

Composting is always described as a genuine aerobic process, in which oxygen plays an important role. During composting and peak heating many of the polysaccharides (cellulose and hemicellulose, also known as pentosans) are broken down and the quantity of lignin – humus complex remains virtually constant (Gerrits, 1988). The

substrate for culturing *A. bisporus* is the most complex culture medium used for edible mushroom production (Sanchez, 2004, Dundar and Yildiz, 2009). Composting is a biological decomposition process, wherein organic matter is degraded to achieve inorganic nutrients and stable organic material (compost) at the end. During the thermophilic phase, high temperatures accelerate the breakdown of proteins, fats, and complex carbohydrates like cellulose and hemicellulose. Then the compost temperature gradually decreases and mesophilic microorganisms once again take over for the final phase" curing" or maturation of the remaining organic matter (Trautmann and Olynciw, 2001).

During the decomposition process, initial carbon to nitrogen ratio (C: N) in compost material is the major controllable factor indicating the digestion process which include enzymatic activity by microorganisms. The C/N ratio can be used as an indicator for compost maturity or fermentation. The initial C/N ratio in compost affects the quality of final products. It was reported that an initial C/N ratio of 25 – 30: 1 is suitable for microbial activities during the nitrification process (Kamolmanit and Reungsang, 2006). However, certain raw and unstable materials (e.g. some manure) may have low C: N ratios while; conversely immature compost with a high ash or low organic matter content could have a similarity low ratio (Trautmann and Olynciw, 2001).

When the carbon to nitrogen ratio is less than 30 to 1, organic matter decomposes rapidly but a loss of nitrogen occurs producing an ammonia gas. If an ammonia odor is present it means valuable nitrogen is being lost in the air. To counter this loss, some high carbon materials should be added to the pile (Trautmann and Olynciw, 2001). Lower C/N ratio increases the loss of nitrogen by leaching (e.g. nitrate mobilization) and ammonia volatilization; whereas higher levels necessitate progressively longer composting time as nitrogen becomes the microbial– limiting nutrient (Trautmann and

Olynciw, 2001). Compost requires plenty of oxygen, along with the right mix of ingredients, to keep decay-causing organisms going strong. These micro organisms need carbon for energy and nitrogen for reproduction (Trautmann and Olynciw, 2001). A low O2 concentration during pasteurization resulted in reduced moisture loss and increased compost bulk density, which influenced the filling weight and mushroom yield per unit of cropping area (Noble and Gaze, 1996).

The physico – chemical properties of compost and the average weight of mushrooms, have a significant relationship (Singh, *et al.*, 2000). Predicted air-filled porosity decreased with increasing moisture content and compost depth for all materials. In general, permeability increased with increasing air-filled porosity and decreasing bulk density (Ahn, *et al.*, 2008).

With higher porosity and water holding capacity, the number and weight of fruit bodies will be higher. In contrast, when porosity was increased and bulk density was reduced, a higher number of fruit bodies with lower weight were recorded. It was also suggested that the number of mushrooms is larger with higher pore spaces, and this has a positive effect on both the yield and mean weight of mushroom (Singh, *et al.*, 2000).

Increasing the compost nitrogen content from 1.6 to 3.1% of the compost dry matter by increasing the quantity of deep litter poultry manure added to straw resulted in a greater yield of mushrooms. Further increases in nitrogen content resulted in an incomplete clearance of ammonia from the substrate and subsequently low or no mushroom yield (Noble and Gaze, 1996).

Organic matter content of the substrates usually ranges from 55.0 to 62.8% based on the assumption that carbon constitutes 58% of the organic matter. Composting works best with a moisture content of 50% (moist but not soggy). Too much moisture slows decomposition and produces an unpleasant odor due to the activity of methane gas

producing micro organisms. Conversely if the pile is too dry, decomposition will be very slow or may not occur at all.

Heat is supplied by the respiration of microorganisms as they break down organic matter. High temperatures favor the microorganisms as rapid decomposers. These microorganisms are most active with temperature around 71^0C and well-built compost bile can maintain that constant temperature. Final compost had a bulk density of 486 kg / m^3 (range 440 – 510 kg /$m^{3)}$ at 70.4 % moisture (range 66 – 75 %), and produced average fresh mushroom yields of 19.4 kg per bed m^2 (range 17.3 – 22.7 kg / m^2), or 0.66 kg per kg dry compost, in a 3 flush- cropping cycles (Miller, *et al.*, 1990).

The average pH of fresh mushroom compost is 6.6. It is clear that a slight alkaline pH of compost had a positive impact in enhancing the yield of mushrooms (Singh, *et al.*, 2000).

2.6. Spawn run:

If phase I and phase II of the composting have been done well, the compost is suitable for the selective growth of mushroom. The spawn must be mixed carefully with the compost, so that the spawn points are distributed as well as possible throughout the compost mass. The rate of 5 liters of spawn (spawn is usually sold on a volumetric rather than on a weight basis, as a general guideline, one liter of spawn weighs about 0.6 kg) (Rinker,1986) per ton of compost will be adequate to achieve a maximum yield. For *A. bisporus*, the optimum temperature during this period (mycelial growth) must be constant from 24 to 26^OC. After 14 days, the compost is completely colonized and can be cased (Rinker, 1986). Noble *et al.*, (2003), said that most *A. bisporus* strains have a requirement for a separate casing layer that has specific physical, chemical and microbiological properties which stimulate and promote the initiation of primordia. Primordia are formed after mycelial colonization of the nutritional substrate and casing

layer (Noble, *et al*., 2003). The CO_2 content in a growing room during mycelial growth must be about 1 to 2 % in the compost and around 0.3 % in the air. The relative humidity should be 90 – 95% (Rinker, 1986). The pH also drops during the mycelial growth in average fall of 0.1 pH unit per day. This drop occurs as a result of producing large quantities of oxalic acid from the mycelium. So the pH of the compost at the time of casing is a good indication of the extent of mycelial growth. In optimum conditions, it should be about 6.5 (Gerrits, 1988, Rinker, 1986, Tawayah, 2008). The compost can be covered by newspaper or plastic sheets (paper should be wetted regularly), which also acts as a physical barrier to flies or disease spores (Rinker, 1986).

2.7. Mushroom contaminants:

Mushrooms as any other living organisms, can be affected by certain pests, which affect the growth of mycelium and decrease its production (Largeteau and Savoie, 2010). Different communities of microorganisms predominate during the various composting phases. Initial decomposition is carried out by mesophilic microorganisms, which rapidly break down the soluble, readily degradable compounds. The heat they produce causes the compost temperature to rapidly rise. As the temperature rises above $40^{O}C$, the mesophilic microorganisms become less competitive and are replaced by others that are thermophilic, or heat – loving. At temperatures of $55^{O}C$ and above, many pathogenic microorganisms are destroyed. Because temperatures over $65^{O}C$ kill many forms of microbs and limit the rate of decomposition, compost managers use aeration and mixing (turning) to keep the temperature below this point (Trautmann and Olynciw, 2001).

If a pest gets into a growing room, it can spread rapidly because of food availability within a relatively small scale. In addition, many pests cannot be controlled using

chemical pesticides, either because there are no products labeled for mushroom use, or because materials don't even exist for a specific type of pest organism.

Although, mushroom production represents one of the best examples in agriculture where insects and diseases can be controlled without the use of pesticides. A proper phase II composting can provide compost, which is selective for the growth of the mushroom fungus, restricting the development of many other competitor fungi. Once the mushroom mycelium begins to colonize the compost, it produces metabolic products, which directly retard the growth of competing fungi and bacteria (Rinker, 1986). In compost, fungi are important because they break down tough debris. They spread and grow vigorously by producing many cells and filaments, and they can attack organic residues that are too dry, acidic or low in nitrogen for bacterial decomposition (Trautmann and Olynciw, 2001).

Green mold disease caused by *Trichoderma aggressivum* is still a major disease in mushroom growing regions, worldwide (Stamets and Chilton, 1983). This mold is considered as an indicator of compost quality. However, some species can reduce both crop yield and quality. The optimum growth temperature ranges between 22 – 26^{O}C. Sporulation can be observed within 10 days of contamination. *Trichoderma* grows particularly well in pH below 6 where the nitrogen value is low. Compost with a high carbon: nitrogen ratio greater than 16:1 at spawning typically favors development of the pathogen. Normal compost at spawning will be about 15:1. *Trichoderma* species are readily found in soil and in organic matter (Rinker, 1986).

Two fungal diseases characterized by anamorphous masses growing in place of sporophores, wet bubble disease (*Mycogone perniciosa*) and dry bubble disease (*Verticillium fungicola*), are world wide in distribution (Largeteau and Savoie, 2010).

The pathogenic fungus *Verticillium fungicola*, responsible for dry bubble disease of the common mushroom (*A. bisporus*), causes various symptoms on its host, bubbles (undifferentiated spherical masses), bent and / or split stipes (blowout) and spotty caps (Largeteau, *et al.*, 2007). The fungal pathogen affects the morphogenesis of fruiting bodies in its fungal host (Largeteau, *et al.*, 2007). The optimum temperature for disease development is about $20^{O}C$. At this temperature, dry bubble and split stem symptoms will develop in 10 – 14 days after inoculation. The contaminated casing material is probably the most common source of *Verticillium* disease (Rinker, 1986).

Wet Bubble disease caused by *Mycogone perniciosa*, can be found from time to time, and it is not a serious economical threat to commercial mushrooms. The disease is best recognized by the large "cauliflower-like" distortion of the mushroom. A mature mushroom may be also attacked. Contaminated casing material is the primary source of the pathogen. The fungus is thought to be common in soils (Rinker, 1986).

Compost formulation is very important, if it is incorrect, excess nutrients will be left in the compost. If nitrogen supplementation is too high, for instance, excess ammonia will be produced. Ammonia is toxic to mushroom mycelium and promotes the growth of undesirable fungi like *Coprinus*. Excess carbohydrates promote the growth of fast-growing competitor organisms like *Aspergillus*, which will overtake mushroom mycelium (Stamets and Chilton, 1983).

3. MATERIALS AND METHODS

All experiments were conducted at the commercial mushroom farm (Pioneer Agricultural Projects Company, located in Almanarah / Aljiza, 35 km south of Amman), during June 2010 to July 2011.

Agaricus bisporus was produced using the following procedure. Mushroom was produced in three cycles (rotations); each cycle took about 3 months. During the three cycles, sampling was done at all stages of mushroom production to predict the most fruitful and profitable production technique. In the farm, in which the experiments were conducted, there were six rooms (13m long × 6m width × 3m height), each room contains two rows of metal boxes, each row consists of 12 boxes with four layers (the total = 96 + 4 = 100 boxes), each box has dimensions of 1.45m ×1.45 m with a depth of 0.20m. The rooms were the same in all conditions except in the compost which filled the boxes and the date of spawning.

Mushroom mycelium grows on a wide variety of plant matter and animal manures. Many of the contaminated organisms directly competed with the mushroom mycelium for the available nutrients and inhibited its growth. By composting, nutrients favored by competitors gradually diminished while nutrients available to the mushroom mycelium were accumulated. With time, the substrate became specific (selective) for the growth of mushrooms.

3.1. Compost preparation

Three replicates (cycles) / treatments were conducted in six growing rooms (used as treatments) with different characteristics. The boxes were covered with newspapers to protect them from drying and evaporation.

The purpose of composting was to prepare a nutritious medium of such characteristics that the growth of mushroom mycelium was promoted to the practical exclusion of competitor organisms.

3.1.1. Pre-wetting

A pre-wetting period was used on straw to break down the waxy layer and soften the plant fibers to permit water retention and utilization by the composting organisms. Wheat straw was chosen carefully and was clean, new, and golden in color and with about 5-8 cm length. Pre-wetting was achieved in a flat or peaked pile on which water was irrigated to the point of run-off. The piles were irrigated with run-off tap- water. The weight of straw for each time of preparation was about 10 tons. The piles were turned over every 2-3 days. This increased the availability of oxygen and provided uniform moisture and decomposition. Run-off water was collected and recycled. This reduced unnecessary nutrient losses, especially when water-soluble organic sources were used, and prevented pollution of the environment. Broiler chicken manure was added to the pile in a ratio of 1:2 manure to straw (w/w), so 5 tons of manure were added.

3.1.2. Compost formulation

The aim of the formula is to achieve a nitrogen content of 1.5 – 1.7 % at the initial make up of the compost pile by adding nitrogen-rich materials (gypsum, urea or ammonium sulphate). In order to the formula to be effective, the moisture content and nitrogen content must be corrected. The following formula was used in the experiments

Wheat straw: 10,000 kg

Broiler chicken manure: 5,000kg

Gypsum ($CaSO_4$): 800 kg

Urea (46% N): 5 kg

Water: 5000 m^3.

3.1.3. Ricking

After the pre-wetting period (which took 2 weeks), additional nitrogen-rich materials were added and ricks (windrows) were formed. The pile was 1.8m in width, 1.8m in height and the length was as required. A two sided box can be used to form the pile (rick). The sides of the pile should be firm and dense, yet the center must remain loose throughout phase I composting. The pile was turned over and the water was added. This period took 7 days until the temperature reached 70-75^{0}C. A linear meter of compost corresponded from 1.0 to 1.7 ton, depending on the width of the rick. Shorter length straw required narrower ricks to prevent compaction and anaerobic conditions. Wider piles were used during the colder winter months. At the end of phase I, the compost had the following characteristics: a) chocolate brown color; b) soft, pliable straws, c) moisture content from 68% to 74 % and d) strong smells of ammonia.

3.1.4. Pasteurization Techniques:

Pasteurization was accomplished early in the phase II operation and was necessary to kill many insects, nematodes, and other pests or pathogens that may be presented in the compost

When the phase I was finished, the compost was entered to the pasteurization tunnel.

The pasteurization tunnel is a long room (13m long), with insulated walls (2.8m width) and (3m high), it has an upper opening to take air out. The floor has a channel covered by steel net underneath the compost, from which the hot steam enters the room from outside. The channel is attached to a strong fan, which is located outside the room to push the hot steam air from the boiler to inside the room (tunnel) which raised the temperature of compost up to 45^{0}C for 3 – 4 days.

The compost should full the tunnel completely, with smooth surface. The tunnel must be closed from two sides, and there are four thermometers inside the compost, other one hanged over the compost from the ceiling and another one was under the compost. The temperature was adjusted by controlling the quantity of air inside the tunnel. In the first stage the quantity of air should be 150 – 180 m^3 air / 1 ton straw, and then the quantity should be raised to 200m^3 air / 1 ton of straw to facilitate the pasteurization.

The quantity of compost inside the tunnel can be calculated according to the following equation:

Quantity of compost in the tunnel = quantity of straw multiply by constant (3.3)

$= 10 \times 3.3 = 33$ ton.

The quantity of air required for composting equals 33 tons × 150 m^3 / ton = 4950 m^3.

The quantity of air can be adjusted by controlling the speed of the fan.

The temperature inside the tunnel rose gradually at a rate of two degrees / hour, until it reached 58.5^{0}C – 60.5^{0}C. At this point, the pasteurization started, and it took about 8 – 10 hours to reach this degree. During pasteurization phase, the temperature must be constant (58^{0}C – 60^{0}C ± 0.5^{0}C) for 8 – 10 hours. At the end of pasteurization period, the boiler was turned off and the temperature allowed decreasing drastically, while the tunnel remained close, and by this step, the compost entered the conditioning phase. The door was opened to allow the fresh air to enter the tunnel, and the temperature started to decrease in a rate of 1.5 – 2 degrees / hour, taking in consideration that the compost must conserve a temperature degree between 45 – 55 ^{0}C which is the favorable temperature for ammonia to be removed, and the temperature must not decrease less than 45^{0}C. During pasteurization period the concentration of ammonia was in its high rate, while in the conditioning phase, the concentration decreased gradually until it reached the lowest degree (less than 0.1%). To get red of ammonia, the temperature was

kept constant at 45 – 55^{0}C for 3 – 4 days, then the temperature decreased up to 25 – 28 ^{0}C (suitable for spawning) by entering more fresh air from outside through the opening.

3.2. Spawning:

Once sterilized wheat grain had a bit of mycelium added to it, the grain and mycelium was shaken 3 times at 4- day intervals over a 14- day period of active mycelial growth. Once the grain was colonized by the mycelium, the product was called spawn. Spawn can be refrigerated for a few months (about 3- 4 months). The compost after preparation was inoculated (spawned) with 600 grams of spawn of *Agaricus bisporus* / box, into boxes of 2.1025m^2 at the rate of 200 kg compost / box. The insecticide "Diazinon granular 60%" was added to the compost before spawning at a rate of 1 kg / 1 ton of straw. The growing boxes were filled with compost at a rate of 200kg compost / box. Spawn was distributed into the compost and mixed thoroughly with the compost. The spawning rate was expressed on the basis of fresh spawn weight per dry compost weight; 600 grams of spawn were added to 200 kilograms of compost (box). Granular Diazinon insecticide was added to the compost before spawning at a rate of 1 kg. / 1 ton straw (10kgs/10tons). The compost temperature was maintained at 24 – 26^{0}C and the relative humidity was kept high to minimize drying of the compost surface and the spawn. Under these conditions, the spawn will produce a thread- like network of mycelium throughout the compost. The mycelium was grown in all directions from a spawn grain, and eventually the mycelium from the different spawn grains fused together appeared as a white mass throughout the compost after fusion had occurred. The spawned compost was covered with papers or plastic sheets to prevent dryness and evaporation, the paper was sprayed with water daily and the temperature was recorded, while the growing room kept tightly closed , to conserve temperature and CO_2.

After 10 – 12 days, the compost was examined by hand to observe the colonization percent of mycelium and to detect any fungal contaminants by observing any green molds grown on the surface of the compost for each room.

3. 3. Casing:

Casing is a top dressing applied to the spawn run compost on which the mushrooms eventually form.

A mixture of peat with ground limestone was used as casing layer.

After 14 days of spawn growth at 24 – 26 ^{0}C, a 3 – 5 cm of casing layer (Toptera / Holland - peat) was added on the top of boxes. To prepare the casing material; the peat was spread on a clean plastic sheet, water and formalin (37%) were added to the peat at a rate of 5 liters + 5 ml formalin / one bag (20 liters) of peat, the peat was mixed with water and turned over several times for 3 days. After three days of casing, the mycelium colonized the casing layer, then water was added at a rate of 2liters / m^2 of bed boxes, then watering continued daily and the total quantity of water added during 7 days was 12 – 14 liters of water / m^2.

The compost temperature was maintained between 24^{0}C – 26^{0}C for 5 – 6 days. Fresh air was then introduced into the growing room to lower the compost temperature to 19 – 20 ^{0}C (air temperature 17^{0}C) and to obtain a CO_2 concentration of 0.09 – 0.12 % (v/v); air temperature and relative humidity were maintained at 16 ± 0.5 ^{0}C and 85 – 90 %, respectively, throughout the remainder of the crop, light did not important for growth of the mushroom. Mushrooms were harvested by hand during 4 first flushes over about 35 – 40 days; with the first flush being picked 21 – 23 days after casing (36 – 38 days from spawning). The number per size category and weight of mushrooms harvested were recorded daily for each treatment. Three successive crops (cycles) were conducted.

3.4. Ruffling:

A widely used practice to totally disturb the mycelium and casing layer. This process supported build-up of CO_2 in the compost and the casing layer, thereby stimulating mycelial growth. Eight days after casing, the newspapers were removed, just before the first mushroom initials were formed, the casing layer was roughened. This step was done to encourage a large and regular first flush and also to restore the structure of the casing material after the damaging effects of the frequent watering. Ruffling was necessary to increase the yield in first flush, and the uniformity of fruiting bodies. It was noticed that ruffling process increased yield but delayed the first flush, because the cutting mycelium needed extra time to cure. Ruffling helped oxygen to enter through the compost and CO_2 to get out.

3.5. Watering:

The water content (moisture) of the compost throughout the spawn run was preserved by covering the boxes with damp newspaper; no water was applied directly to the compost. The water content of the casing materials was raised to field capacity in the first 3-4 days after casing. Field capacity was maintained by watering lightly every two days until pin initiation, (day 12). At this time irrigation was stopped until the mushrooms were approximately 5 mm in diameter, in order to avoid damaging the developing mushroom initials. A similar watering pattern was followed for subsequent flushes.

3.6. Temperature:

Vegetative mycelial growth was encouraged by maintaining the bed (compost) temperature at 26 ^{0}C ± 1 ^{0}C. Heat produced by biological activity in the compost was met by gradually decrease of the air temperature from 26 ^{0}C to 22 ^{0} C through cooling system.

During the first stages of pin formation, (day 13), the air temperature was decreased up to 16 ^{0}C. These temperatures were maintained until the end of the cropping cycle.

3.7. Ventilation:

Ventilation was essential for growing mushroom, and it was also necessary to control humidity and temperature. Formation of tiny mycelial aggregates on the surface of the casing layer on days 12 and 13 provided the means of recognizing the correct time to ventilate with fresh air which was commenced on day 13 (at the same time as the temperature drop) and continued throughout the cropping period.

3.8. Humidity:

Relative humidity (RH) was kept as high as was possible, for the entire cropping trial by ensuring the floor and walls were kept wet. The relative humidity was maintained at a fairly constant 90%, however after day 13, when ventilation commenced, the relative humidity fluctuated between 80% and 90% in response to the heating and cooling of the ventilating unit. Relative humidity was measured daily (at 10 o'clock in the morning), using a thermo hydrometer.

3.9. Harvesting and determination of yield:

Four flushes of mushroom were collected during the production cycle. Each cycle lasted for about three months (75 – 100 days), depended on the productivity. The production was recorded for four cycles in six growing rooms, so the six rooms represented six replicates, and the four cycles represented four experiments, and the average yield was taken.

It took approximately 14 weeks (3 – 4 months) to complete an entire production cycle, from the start of composting to the final steaming off after harvesting had finished.

Mushrooms were harvested in a 7-10- day cycles, but this may be longer or shorter depending on the temperature, humidity, and cultivar. A shorter harvesting time allowed

more crops to be produced in a year and helped to prevent disease and insect problems. When mature mushrooms were picked, an inhibitor to mushroom development was removed and the next flush moved toward maturity. Mushrooms were picked at a time when the veil was not too far extended.

Mushroom maturity was evaluated by how much of the veil is open, not by size. Mature mushrooms were both large and small. Mushrooms were harvested at the point where the veil was just beginning to rupture (i.e. in the button stage, (cap tightly closed) and the cup stage, (veil broken)). Picking of the mushrooms was done with a gentle twisting motion. If there was any danger of damaging the surrounding mushroom initials using this technique, the sporocarps were trimmed off with a knife.

Mushrooms were harvested, counted and weighed daily. Before weighing, any adhering casing material at the base of the mushroom was removed with a sharp knife. At the end of 4^{th} flush, yield, productivity and biological efficiency (BE) were determined and average mushroom size was calculated as fresh mushroom weight divided by the number of mushrooms harvested. Biological efficiency was determined as the ratio of fresh mushrooms harvested in kg per dry substrate weight in tons, and expressed as a percentage. Yield was expressed as kg / m^2. Diseased, malformed, and fly-damaged mushrooms were discarded, and should not be touched.

The composting and mushroom production cycle procedure can be summarized as follow:

Day	procedure
-13	break bails of straw and add water
-8	turn straw and add water
-5	add chicken manure
-2	turn and add water and N-supplements (urea)

0	phase I
2	Turn stack, adding gypsum (80 – 85kg/ tone straw).
5	turn stack, adding water
6 / 7	phase II, pasteurization & conditioning
10	spawning
24	casing
32	ruffling
48	harvesting

3.10. Production parameters:

3.10.1. Biological efficiency (BE) % = $\frac{\text{fresh wt. of mushrooms (FWM)}}{\text{dry wt. of compost (DWC)}} \times 100$

B E% = F W M / D W C × 100

F W M was determined at the end of harvesting (fourth flush).

F W C was determined at the end of phase II composting.

3.10.2. Productivity (P) = $\frac{\text{Fresh weight of mushroom (FWM)}}{\text{Fresh weight of compost (FWC)}} \times 100$

Productivity was expressed as the percentage (ratio) of the fresh weight of harvested mushrooms over 100 kg of compost fresh weight.

P = F W M / F W C × 100.

3.10.3. Uniformity:

Mushrooms with pileus diameter less than 4 cm (with close or open caps) were counted and sorted into the size category "Baby mushroom" (BM) which usually weighed about 10 grams (one kilograms contains around 100 fruits), and those with pileus diameter more than 10 cm into the category "Giant mushroom" (GM) which usually weighed more than 50 grams per fruit. The mushrooms with pileus diameter between 4 – 5 cm,

into the category “Normal” (NM), and usually weighed about 15 grams per fruit, the mushrooms with pileus size between 5 – 7 cm into the category “Medium” (MM), and this usually weighed about 20 grams per unit, while the mushrooms with pileus between 7 – 10cm in the category "Large" (LM), and usually weighed about 30 – 35 grams per fruit. In addition, the average weight of normal, medium and large sporophores was calculated.

Average mushroom weight showed the tendency to decrease as yield increased.

3.11. Physical and chemical analysis of the substrate:

During production period, the compost was analyzed in every growth stage. Substrate samples were taken from phase I, phase II composting, after pasteurization, after spawning, after casing and after each flush of mushroom. The compost was analyzed for physical properties; bulk density, porosity, chemical properties; pH, moisture content, dry matter, organic matter content, ash, total organic carbon, nitrogen content and the C/N ratio. The relationship between these properties and the production (weight, size and uniformity) and productivity (yield of mushroom in kg divided by weight of substrate on dry bases) were demonstrated. In addition, the fungal contaminants, which affected and / or competed with the mushroom, were identified and the effect of these contaminants on the yield was demonstrated.

Homogenized samples of the compost were taken from each stage of growth to monitor the proposed changes in the physical, chemical and microbiological properties. All samples were analyzed in triplicate and results were averaged. The samples were used on dry weight basis for chemical analysis. Physico-chemical properties were determined using methods suggested by various workers: bulk density (Blake, 1965), porosity (Allen, 1974), pH (Jackson, 1967), organic matter, total organic carbon (TOC), total nitrogen were evaluated by Kjeldahl method (AOAC, 1975).

Bulk density is the weight per unit volume of oven dry compost, which is commonly expressed as grams per cubic centimeters (Foth, 1984).

Bulk density (BD) determination was perforated by mixing and grinding the compost sample, then sieving through 1.6 mm sieve and weighing the compost. Placing the sample in a graduated cylinder and measuring the volume.

Particle density is given only to solid particles, and it does not vary with the amount of spaces between particles. It is defined as the mass (weight) per unit volume of compost particles (compost solids), and it is frequently expressed as grams per cubic centimeters (Foth, 1984).

Particle density: A clean dry 100ml volumetric flask was weighed, 50 g of oven dry compost placed in it, and reweighed. Approximately 50ml of water was then poured into the flask and the contents boiled gently for 2 – 3 minuets to remove any trapped air in the sample. After cooling at room temperature, cold boiled distilled water was added to the flask up to the 100ml mark and then reweighed. Finally, the contents of the flask were washed out and the flask filled with cold, boiled distilled water to the 100 ml mark and weighed. The particle density was calculated by dividing the weight of compost by the weight of water displaced by the compost material (Allen, 1974, Blake, 1965).

The percentage of total porosity was calculated after determination of bulk and particle density using the relationship:

$$\text{Porosity \%} = \frac{\text{particle density - bulk density}}{\text{particle density}} \times 100$$

Total nitrogen was determined by the micro-kjeldahl method (Rapid distillation unit, LABCONCO, made inn England) according to Official methods of analysis (AOAC, 1975). In this method 0.5 grams of compost (on dry weight basis) was digested in

concentrated sulfuric acid by (Tecator Digester System No. 6, 1007 digester, made in Sweden) to break down the organic matter and to reduce the nitrogen as ammonia. Then, the ammonia liberated by boiling with sodium hydroxide (NAOH) and the produced steam was distilled into boric acid to form ammonium borate. Finally, the ammonium borate was titrated with standardized acid solution 0.1N HCL.

The moisture content (MC) was determined by drying the samples at 105^0C overnight in an electrical oven (Gallenhamb oven, made in England). By weighing 100 g of fresh compost and place them in a crucible. The crucibles were placed in the oven at 105^0C for 24 hrs. then the samples were removed from the oven and put in the desiccator until they dry. Samples were weighed to calculate the moisture content using the following equation:

MC% = (Wet weight – dry weight) / wet weight × 100.

The pH was determined by using a double- distilled water suspension of each compost sample in the ratio of 1:10 (w/v).

Hydrogen ion concentration (pH): A 50ml beaker was half filled with an equal volume of fresh compost and distilled water. The solution was stirred for 2-3 min then allowed to stand for 30 min before measuring the pH with a pH-meter (Adwa-AD100, Romania)

Ash content determination: The weighed sample (0.5g) was ignited at 550^0C for 6 – 24 hr. in a muffle furnace (Naber Industrieofenban D-2804 Lilienthal / Bremen, made in W.Germany) to burn the organic matter. The remaining material (ash) was weighed (after drying in a desiccator) and its percentage was calculated as follows:

Volatile solids % (TOM) = (dry wt. – ash wt.) / dry wt. × 100.

Total organic carbon (TOC) % = 1.703+ 0.52(TOM %).

Ash % = (wt. at 550^0C / wt. at 105^0C) × 100.

Organic matter % = 100 – (MC + Ash).

Ammonia concentration: 5 grams of compost sample were taken and placed in a serum flask with 100ml of potassium chloride (KCL) solution, the solution was shaken for 30min and then centrifuged for 10 – 15 min.10 ml sample was placed in distillation flask with 1.2 g magnesium oxide (MGO) to increase its alkalinity to ph 10, so that only organic nitrogen was analysed.10 – 25 ml of Boric acid was placed in an Erlenmeyer flask for distillation collection. The distillate was titrated with 0.02N H2SO4.

Calculation of ammonia % =

$$\frac{\text{Acid volume} - \text{blank volume} \times \text{acid normality} \times 17}{1000} \times \frac{\text{dilution factor}}{\text{sample wt.}} \times 100$$

3.12. Estimation of fungal contaminants:

The dilution plate technique was used for estimating fungal populations contaminating the substrate. About 10 g fresh weight sample of compost was placed in 250 ml Erlenmeyer flask containing 90 ml sterile distilled water (SDW). The mixture was shaken at 140 rpm in a Gallen Kamp orbital shaker for 30 min. (Cooke and Flegg, 1962). Ten – fold serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) were prepared. One ml of each dilution was spread on a Petri dish containing potato dextrose agar (PDA) medium and the cultures were incubated at $25^{O}C$ for 5-7 days in darkness. Fungi were identified using their morphological and cultural characteristics.

$$\text{Colony forming unit (CFU)/ml} = \frac{\text{number of colonies per 1ml}}{\text{Oven dry weight of sample}} \times \frac{1}{\text{dilution}}$$

All compost samples were tested in Petri dishes on potato dextrose agar (PDA). The incubation was carried out at 25 ^{O}C and the growth of fungal colonies was observed. The identification of the isolated fungi was done on the basis of macroscopic and microscopic characteristics. Specimens were stained in lacto phenol, and taxonomical keys were used for identification of the fungi.

The mushroom yield (weight, uniformity & size of fruiting bodies) was recorded for each flush in each growing room. Also, the earliness of production was taken in consideration (the time elapsed between the day of spawning and the day of first flush of harvesting), and the flushing scheme (the spreading of the yield over the successive harvest weeks). All steps were photographed.

Statistical analysis:

Data in this study were analyzed and all values were presented as the mean ± standard deviation (SD).Differences between different means were compared using the least significant difference (LSD) test.

4. RESULTS

4.1- Effect of compost pH during crop cycle of mushroom (*Agaricus bisporus*) on yield and productivity.

The pH of the compost ranged between 6.52 and 7.75 during crop cycle (Table 1), and it decreased gradually as the mycelium of the mushroom grown in its life stages. The pH did not affect the yield and productivity significantly when it was in the normal range (6.5 – 7.5), but it was noticed that when the pH of the compost was increased more than 8.0, as shown in Table 1, the growth was severely affected.

Table 1: Effect of pH of compost on growth and yield of *A. bisporus*.

Room No.	composting		Spawning	Casing	1st flush	2nd flush	3rd flush	4th flush	Total production (kg)
	Phase I	Phase II							
1	*7.20 A	7.18 A	7.16 A	6.80 B	6.68 C	6.55 C	6.66 C	6.57 C	5533.8
2	7.75 A	7.53 A	6.94 A	6.67 B	6.65 C	6.58 C	6.66 C	6.62 C	5140.7
3	7.10 A	7.00 A	6.97 A	6.97 A	6.68 C	6.55 C	6.66 C	6.57 C	5744.1
4	7.10 A	7.20 A	7.16 A	6.89 A	6.78 C	6.66 C	6.54 C	6.55 C	2465.6 *
5	7.26 A	7.40 A	7.74 A	7.20 A	6.52 C	6.55 C	6.60 C	6.57 C	4400
6	8.07 D	7.94 D	8.10 D	8.04 D	---	---	---	---	--- ***

*Means of pH values of three production cycles.

** The reduction of yield was as a result of *Fusarium* disease.

*** The loss of yield was as a result of *Rhizoctonia* disease.

Means with the same letter are not significantly different.

4.2- Effect of bulk density and porosity of compost on yield and productivity of mushroom (*Agaricus bisporus*).

The bulk density of the compost ranged between 0.58 at the beginning of the composting process and 0.78 at the end of the crop cycle (Table 2). It was noticed that the bulk density increased as the mushroom mycelium grown. In contrast, the porosity of the compost decreased with time, and it was ranged between 96% and 87%, and it was noticed that when bulk density increased the porosity decreased, and vice versa. The bulk density means the mass of certain volume of compost, where the porosity means the percentage of pore space inside the compost particles, and the two parameters affected significantly yield and productivity as shown in Table 2. Although it was noticed from the experiments that when the porosity increased (less bulk density), the yield was increased and when the porosity decreased (high bulk density) the yield decreased (as in room number four) as a direct result.

Table 2: Effect of bulk density / porosity (BD/ porosity) of compost on growth and yield of *A. bisporus*.

Room No.	composting		Spawning	Casing	1st flush	2nd flush	3rd flush	4th flush	Total production (kg)
	Phase I	Phase II							
1	0.65/0.95 d/B	0.66/0.94 d/B	0.66/0.94 d/B	0.7/0.92 c/D	0.72/0.91 b/E	0.75/0.9 a/E	0.76/0.89 a/E	0.78/0.87 a/E	5533.8
2	0.60/0.96 f/A	0.64/0.95 e/B	0.66/0.94 d/B	0.68/0.93 c/C	0.71/0.92 b/D	0.72/0.91 b/E	0.72/0.91 b/E	0.75/0.89 a/E	5140.7
3	0.58/0.96 f/A	0.60/0.96 f/A	0.65/0.96 d/A	0.66/0.94 d/B	0.68/0.93 c/C	0.69/0.93 c/C	0.69/0.92 c/D	0.72/0.91 b/E	5744.1
4	0.62/0.96 f/A	0.65/0.95 d/B	0.70/0.93 c/C	0.72/0.91 b/E	0.75/0,89 a/E	0.77/0.89 a/E	0.75/0.9 a/E	0.78/0.89 a/E	2465.6
5	0.66/0.94 d/B	0.67/0.94 d/B	0.68/0.93 c/C	0.70/0.92 c/D	0.72/0.91 b/E	0.75/0.89 a/E	0.71/0.93 b/C	0.72/0.91 b/E	4400
6	0.64/0.95 e/B	0.71/0.94 b/B	0.72/0.92 b/D	0.77/0.88 a/E	---	---	---	---	---

4.3- Effect of moisture content (MC) and dry matter (DM) of compost on yield and production of mushroom (*A. bisporus)*

The moisture content was ranged between 50% and 72%, and it was decreased during the crop cycle as the mushroom mycelium consumed water from the compost and casing layer, with some fluctuating as a result of irrigation process (Table 3). In the other hand, the dry matter decreased as a result of degradation and utilization of organic matter by the microorganisms, and it was ranged between 50% and 28%. As it was clear in room No. 6, where MC/DM were 50/50 in all phases and casing period, the yield was zero.

Table 3: Effect of moisture / dry matter (*MC/ DM) of compost on growth and yield of *Agaricus bisporus*.

Room No.	Composting Phase I	Composting Phase II	Spawning	Casing	1st flush	2nd flush	3rd flush	4th flush	Total production (kg)
1	69/31 a/ C	62/38 ab/B	56/44 c/A	60/40 bc/A	52/48 c/A	60/40 bc/A	50/50 c/A	60/40 bc/A	5533.8
2	68/32 a/C	66/34 a/C	62/38 ab/B	57/43 c/A	54/46 c/A	52/48 c/A	50/50 c/A	60/40 bc/A	5140.7
3	65/35 a/C	60/40 bc/A	60/40 bc/A	60/40 bc/A	60/40 bc/A	60/40 bc/A	50/50 c/A	60/40 bc/A	5744.1
4	72/28 a/C	68/32 a/C	66/34 a/C	60/40 bc/A	60/40 bc/A	65/35 a/C	60/40 bc/AB	62/38 ab/B	2465.6
5	66/34 a/C	67/33 a/C	63/37 ac/C	62/38 ab/B	61/39 b/AB	60/40 bc/A	60/40 bc/AB	60/40 bc/A	4400
6	50/50 c/A	50/50 c/A	50/50 c/A	50/50 c/A	---	---	---	---	---

* MC: moisture content percentage, DM: dry matter content percentage.

** Small letters for moisture content, capital letters for DM.

Means with the same letter are not significantly different.

4.4- Effect of organic matter (OM) and ash of compost on yield and production of mushroom (*A. bisporus*).

The organic matter content of compost ranged between 50% and 69%, and it decreased with time as a result of degradation and carbon utilization by the mushroom mycelium during growth as shown in Table 4. In the other hand, ash content remained constant or increased during the life cycle of mushroom growth (Table 4) it affected indirectly yield and productivity, because when the organic matter increased, the porosity increased while the bulk density decreased. Ash content did not affect the yield and mushroom productivity significantly as it was clear in rooms No.3 and No. 4.

Table 4: Effect of organic matter / ash (*OM/Ash) of compost on growth and yield of *A. bisporus*.

Room No.	composting		Spawning	Casing	1st flush	2nd flush	3rd flush	4th flush	Total production (kg)
	Phase I	Phase II							
1	*58/42 b/AB	58/42 b/AB	58/42 b/AB	56/44 c/A	57/43 bc/A	59/41 ab/B	55/45 c/A	53/47 c/A	5533.8
2	59/41 ab/B	58/42 b/B	56/44 c/A	58/42 b/AB	56/44 c/A	56/44 c/A	55/45 c/A	50/50 c/A	5140.7
3	66/34 a/B	69/31 a/B	57/43 c/A	58/42 b/AB	59/41 ab/B	59/41 ab/B	55/45 c/A	53/47 c/A	5744.1
4	67/33 a/B	68/42 a/AB	58/42 b/AB	58/42 b/AB	57/43 c/A	56/44 c/A	55/45 c/A	53/47 c/A	2465.6
5	58/42 b/AB	59/41 ab/B	59/41 ab/B	58/42 c/AB	57/43 c/A	59/41 c/B	56/44 c/A	53/47 c/A	4400
6	57/43 c/A	54/46 c/A	55/45 c/A	55/45 c/A	---	---	---	---	---

* Organic matter (OM) / ash percentage.

** Small letters for organic matter, capital letters for ash.

Means with the same letter are not significantly different.

4.5- Effect of carbon to nitrogen ratio (C/N) of the compost on yield and production of mushroom (*A. bisporus*).

The carbon to nitrogen ratio (Table 5), ranged between 10 and 22, and it was decreased during the growth of mushroom mycelium. The C: N ratio of the compost had a

significantly negative effect on yield and productivity of the mushroom as it was clear in room N0. 4.

Table 5: Effect of (C: N) ratio of compost on growth and yield of *A. bisporus.*

Room No.	**Composting** Phase I	Phase II	**Spawning**	**Casing**	**1st flush**	**2nd flush**	**3rd flush**	**4th flush**	**Total production (kg)**
1	*13.3 B	13 B	12.5 B	14 AB	12.7 B	16.3 A	14.7 AB	12.4 B	5533.8
2	11.2 B	14 AB	15 A	13 B	12.5 B	12 B	15 A	11 B	5140.7
3	14 AB	15 A	16 A	16 A	13 B	16 A	15 A	12.5 B	5744.1
4	22 A	20 A	12.5 B	13 B	11 B	12.5 B	12 B	11 B	2465.6
5	13 B	15 A	13 B	12.5 B	12 B	16 A	13 B	12 B	4400
6	12.5 B	10 B	11 B	11 B	---	---	---	---	---

* Mean values of carbon to nitrogen (C/N) ratio.

**Means with the same letter are not significantly different.

4.6- Effect of fungal contamination on yield and productivity of mushroom (*A. bisporus*).

Eight fungi were isolated from the compost during all stages of production cycle, four of them were saprophytes. Others were very aggressive especially when they appeared early in the season (e.g. before harvesting), some of them were able to cause 100% loss of production as *Rhizoctonia solani* when it appeared during spawning (Table 6).

The eight fungi were:

1- *Rhizopus stolonifer* (bread mold); Class: Zygomycetes, order: Mucorales. (Figures: 1 & 2).

2- *Penicillium digitatum* (*p. etalicum* and *p. digitatum*); Class: Fungi Imperfecti, Order: Moniliales. (Figures: 3 & 4).

3- *Mycogone perniciosa* (Wet bubble disease, white mushroom mold); Class: Fungi Imperfecti, Order: Moniliales. (Figures: 5 & 6).

This mold is named in reference to the tendency to parasitize the mushroom fruit body. Very common, infecting the mushroom itself and causing significant yield losses. One way in which *Mycogone* was carried on to the farm was in the casing material. It occurs naturally in compost from which this aggressive contaminant attacks the mushroom fruit body. It will not grow well at low temperatures(less than $20^{O}C$). It appeared as a whitish mold attacked primordia and turned them into a soft whitish ball of mycelia.

4- *Fusarium moniliforme* (yellow rain mold); Class: Fungi Imperfecti, Order: Moniliales. (Figures: 7, 8 and 9).

It is a fast growing fungus with whitish cottony mycelium. Even a moderate infestation by this contaminant inhibited mushroom growth. Mushrooms affected with this disease, had small caps, and brownish stems. Grain was the main source of *Fusarium* contamination in mushroom culture.

5- *Trichoderma harzianum* (green mold); Class: Fungi Imperfecti, Order: Moniliales (figures: 10 & 11).

A number of green moulds were occurred in the compost and / or on the casing material in certain conditions. The presence of green moulds in the compost was an indicator that the fermentation process or the pasteurization and conditioning of the compost were not perfect. *T. harzianum* was very common in compost and casing layer, parasitized

mushrooms under cultivation and inhibited or reduced fruiting. It was spread during harvesting, bed cleaning or watering. The fungus appeared as

Cottony mold grew in circular colonies on the casing layer or on compost.

6- *Cladosporium herbarum* (dark green mold); Class: Fungi Imperfecti, Order: Moniliales (figures: 12 & 13).

It was the most predominant genus of all the airborne contaminants.

7- *Aspergillus niger* (black mold); Class: Fungi Imperfecti, Order: Moniliales (Figures: 14 & 15). It is found on most any organic substrate, and it preferred a neutral and slightly alkaline conditions (more than 7.0).

8- *Rhizoctonia solani* (White mold); Class: Basidiomycetes, Order: Cantharellales (figures: 16 & 17).

Table 6: Effect of fungal competitors in compost on growth and yield of *A. bisporus*.

Room No.	Phase I	Phase II	Spawning	Casing	1st flush	2nd flush	3rd flush	4th flush	CFU
1								*Rhizopus sp.* *Penicillium sp.*	62.5 50
2				*Rhizopus sp.* *51			*Mycogone sp.* *44.4	*Cladosporium sp.* *175	
3	*Rhizopus sp.* *171			*Trichoderma sp.* *300				*Aspergillus sp.* *25	
4			*Aspergillus sp.* *22 *Trichoderma sp.* *84				*Fusarium sp.* *50	*Rhizopus sp.* *131.5	
5	*Trichoderma sp.* *300		*Trichoderma sp.* *54	*Rhizopus sp.* *30				*Penicillium sp.* *27.5	
6			*Rhizoctonia sp.* *320						

* CFU: colony forming units / ml = No. of colonies/ dry wt. of sample × 1/ dilution

4.7- Effect of ammonia content:

Ammonia was found in compost during phase I composting, since it reached the highest concentration (full mark on the ammonia indicator), then it decreased to the lowest level (≤ 0.05 %) through conditioning process at the end of phase II composting. When the concentration of ammonia was high (≥ 0.1 %), this was an indicator that the pasteurization process was not correctly done.

In our experiment, the concentration of ammonia was ranged from 0.021 up to 0.10 %. Moreover, it was measured at the end of phase II composting (Table 7).

The lowest ammonia content 0.0216 was in room number 3 which resulted with the highest yield and productivity, while the highest concentration of ammonia 0.1998 was in room number 6 which produced nothing (yield equal zero). Ammonia concentration more than 0.05% was lethal for mushroom mycelium. Ammonia concentration in the compost was the limiting factor for mushroom production. The yield was negatively correlated with ammonia concentration during phase II.

Table 7: Effect of Ammonia concentration in the compost at the end of phase II composting on yield and productivity of mushroom (*A. bisporus*).

Room No.	**Ammonia con. %**	**Stage**	**Yield (kg)**	**Productivity**
Room 1	0.02250	Phase II composting	5533.8	27.7
Room 2	0.02558	Phase II composting	5140.7	25.7
Room 3	0.02160	Phase II composting	5744.1	28.7
Room 4	0.09816	Phase II composting	2465.6	12.3
Room 5	0.08610	Phase II composting	4400	22
Room 6	0.19980	Phase II composting	00.00	00.00

4.8- Yield stages & productivity

Amount of yield produced was ranged between 5744.1 kg in room No. 3, and 2465.6 kg in room No. 4, with exception of room No. 6 which produced no yield (Table:8) The highest yield 5744.1 kg was in room No. 3, then room No. 1 came in the second stage and room No. 2 in the third category with quantity of yield equal 5533.8 kg and 5140.7 kg, respectively. In the other hand, room number six had no production (zero kg.), while room No. 5 produced more than room No. 4, with quantities equal 4400 kg and 2465.6 kg, respectively. Also productivity was less in rooms No.4 and N0. 5 compared to the first three rooms. In spite that the growing area and quantity of compost for all rooms were the same.

Table 8: production & productivity of growing rooms.

Room No.	Flush 1	Flush 2	Flush 3	Flush 4	Yield (kg)	Productivity (P)
Room 1	1563.4 A	1249.5 A	950.9 B	1770 A	5533.8	27.7
Room 2	976.3 B	940.5 B	800.3 B	2423.6 A	5140.7	25.7
Room 3	1585 A	1395.3 A	1033 B	1730.8 A	5744.1	28.7
Room 4	682.6 B	950.9 B	320.1 B	512 B	2465.6	12.3
Room 5	729 B	895.5 B	1245.5 A	1530 A	4400	22
Room 6	00.00	00.00	00.00	00.00	00.00	00.00
Total	**5536.3**	**5431.7**	**4349.8**	**7966.4**	**23284.2**	
Percent %	**23.8**	**23.3**	**18.7**	**34.2**	**100**	

* Means with the same letter are not significantly different.

Productivity (P) = $\frac{\text{fresh weight of mushroom} \times 10}{\text{Fresh weight of compost}}$

Table 9: total production, yield, productivity and biological efficiency of white button mushroom (*A. bisborus*).

Room No.	* Total production (kg)	**Yield Kg / m^2	***Productivity FWM/ FWC	# Biological efficiency FWM / DWC
1	5533.8	32.94	27.7	0.66
2	5140.7	30.60	25.7	0.57
3	5744.1	34.20	28.7	0.72
4	2465.6	14.67	12.3	0.32
5	4400	26.20	22	0.55
6	00.00	00.00	00.00	00.00

* Total production= summation of four flushes.

** Yield = weight of fresh mushroom (kg) / area of growing room (m^2).

*** Productivity = weight of fresh mushroom / weight of fresh compost.

Biological efficiency (BE) = weight of fresh mushroom / dry weight of compost (ton).

4.9- Earliness and dry matter of fruiting bodies of mushrooms in different flushes.

Dry matter contents of fruiting bodies of both caps and stems of mushrooms during four flushes were determined (Table: 10). It was ranged between 8.06 and 9.6, and there were no significant differences between different flushes, and also there were no significant difference between caps and stems.

Table 10: Dry matter percentage of fruit bodies of mushroom in different flushes of three rooms.

Room No.	Flush	Yield (Kg)	Earliness (Days)	Dry matter %	
				Cap	Stem
1	First	1563.4 A	36 A	8.50 B	8.9 A
	Second	1249.5 A	8 C	8.06 B	8.8 A
	Third	950.9 B	10 B	8.80 A	9.6 A
	Forth	1770 A	14 B	8.40 B	8.8 A
3	First	1585 A	36 A	8.80 A	8.3 B
	Second	1395.3 A	7 C	8.80 A	8.5 B
	Third	1033 B	10 B	8.60 AB	8.4
	Forth	1730.8 A	10 B	8.50 B	8.6 AB
5	First	729 B	39 A	8.60 AB	8.7 A
	Second	895.5 B	8 C	8.40 B	8.5 B
	Third	1245.5 A	7 C	8.70 A	8.4 B
	Forth	1530 A	7 C	8.50 B	8.8 A

4.10- Brown strain (Portobello) of *A. bisporus*:

Four boxes were spawned with brown strain (Portobello) of *Agaricus bisporus,* in room number five and replicated three times. The area of production was about 8.4 m^2, and the quantity of compost which was spawned was about 800 kilograms, which needed about 2.5 kg of spawn (Table: 11).

Brown strain of *Agaricus* which is known in the market with the name Portobello was not popular as white strains. This strain was more tolerant to unsuitable environmental conditions such as temperature, relative humidity and diseases, and it has more production and productivity than the white strain.

Table: 11: Production of brown strain (Portobello) of *A. bisporus*.

No. of boxes	Area (m^2)	Quantity of compost (kg)	No. of fruits	Average fruit wt. (gm)	Average cap size (cm)	Total yield (kg)	Productivity * (P)
4	8.4	800	2714	70	10	190	22.6
4	8.4	800	2329	88	12	205	24.4
4	8.4	800	2304	92	13	212	25.2
Average			2449	83	11.6	202	24

$$\text{* Productivity (P)} = \frac{\text{fresh weight of mushroom}}{\text{Fresh weight of compost}} \times 100$$

Table 12: Comparison between white & brown strains of *A. bisporus*.

Property	No. of fruits / m^2	Average fruit wt. (grams)	Average cap size (cm)	*Yield Kg / m^2	**Productivity Fwm / fwc	#Average B E
White strain	1108	25	5 - 7	27.7	23.3	0.58
Brown strain	291.5	83	10 - 25	24	25.2	0.63

* Yield (kg) = fresh weight of mushroom / area of growing room (m^2).

** Productivity = fresh weight of mushroom (kg) / fresh weight of compost (kg).

Biological efficiency (BE) = fresh weight of mushroom / dry weight of compost.

5- Discussion:

Mushroom cultivation presents an economically important biotechnological industry that has been developed all over the world. It is estimated that more than 10 million metric tons of edible and medicinal mushrooms were produced in 2004 in different countries (Royse, 2005). Mushroom production can convert the huge lignocellulosic waste materials into a wide diversity of products (edible, medicinal food, feed and fertilizers). However, the majority of cultivated mushroom are saprophytic mushrooms, which live on dead organic matter such as dead trees, straw, compost, etc. White button mushroom (*Agaricus bisporus*), is the most cultivated and consumed mushroom worldwide.

The basic raw materials that can be used for composting were cereal straw from wheat, rye, oat and barley. Of these, wheat straw was preferred due to its more resilient nature. This characteristic provided good structure to the compost. Straw provides compost with carbohydrates, the basic foodstuffs of mushroom nutrition. Wheat straw contained 36% cellulose, 25% pentosan and 16% lignin. Cellulose and pentosan are carbohydrates which upon break down yield simple sugars. These sugars supply the energy for microbial growth. Lignin, a highly resistant material also found in the heartwood of trees, is changed during composting to a "Nitrogen – rich – lignin – humus - complex", a source of protein.

Environmental conditions play a crucial role in the decision whether fruiting bodies will be formed. Production of the vegetative mycelium usually occurs over a wide range of temperatures. Other parameters of fruiting body initiation and maturation include; relative humidity (RH), salinity and pH, moisture content, bulk density and pore spaces, Carbon to Nitrogen ratio(C/N ratio), ammonia concentration, and also the contaminants of competitor fungi.

5.1- Effect of pH of compost:

Tajbakhsh, *et al.* (2008) studied the PH of mushroom compost, and found that PH was reduced from 7.23 ± 0.09 to 6.69 ± 0.01. This shift of pH from the initial near neutral towards acidic conditions could be attributed to the bioconversion of the organic material into other various intermediate species of the organic acids. It has been also reported that the lower pH in the final products might have been due to the production of CO_2 and organic acids by microbial metabolism during decomposition of the substrate (Tajbakhsh, *et al.*, 2008). Our results were in agreement with Tajbakhsh results, where the pH of compost varied during the crop cycle and it ranged between 7.8 and 6.5, so it decreased gradually as the mushroom mycelium grown. The pH of the compost was an indicator of the mycelium health; when the mycelium grew naturally the pH decreased gradually, but when the pH increased more than 8, the mycelium was not grown correctly. Acidity increased, as a result of organic acids production, as oxalic acid from the mushroom mycelium. The highest pH (7.8) was in phase I, in room number 2. The lowest pH (6.52) was in first flush in room number 5. Mycelial growth was less affected by pH, but fruiting body development occurred best at neutral or slightly acidic pH values around 6 -7 and this was in agreement with that found by Fan et al, 2000, and levanon, et al., 1988.

The pH of compost did not directly affect yield and/ or productivity but it was an indicator for any uncorrected action or process or fungal contamination.

5.2- Effect of bulk density & porosity:

Final compost had a bulk density of 486 kg / m^3 (range 440 – 510 kg /m^3) at 70.4 % moisture (range 66 – 75 %), and produced average fresh mushroom yields of 19.4 kg per bed m^2 (range 17.3 – 22.7 kg / m^2), or 0.66 kg per kg dry compost, in a 3 flush-cropping cycles(Miller, *et al.*, 1990).

Our results were higher than that of Miller, *et al*, (1990), so in this experiment, bulk density ranged between 0.58 and 0.78 (580 kg / m^3 – 780 kg / m^3) and it may be as a result of decreasing oxygen, A low O_2 concentration during pasteurization resulted in reduced moisture loss and increased compost bulk density, which influenced the filling weight and mushroom yield per unit of cropping area (Noble and Gaze, 1996).

And it was increased with time. The lowest bulk density (0.58) was in phase I in room number three, while the highest B.D. (0.78) was in 4^{th} flush of yielding in rooms number 1 & 4.

The porosity ranged between 88 and 96, and it was decreased with time. The lowest porosity (88) was in fourth flush in room number 1, while the highest porosity (96), was in phase I compost in rooms 2 & 3 and in phase II composting in room 3.

5.3- Effect of moisture & dry matter:

Moisture content ranged from 50% up to 72%, and it was decreased with time during the crop cycle. The lowest moisture (50%), was in the 3^{rd} flush in rooms 1, 2 and 3 which gave the highest yield, with exception of room number 6, while the highest moisture content (72%), was in phase I composting, in room number 4 which gave the lowest yield (with exception of room number 6). High humidity (90 – 95%) was favorable for pinning and fruiting body initiation, and this was less than what was reported by Fan, *et al.*, 2000.

Dry matter (D. M.) was ranged from 28% up to 50%, and it was increased with time. The lowest D.M. (28%) was in phase I in room number 4, which gave the lowest yield,

while the highest D. M. (50%), was in third flush in rooms 1, 2 and 3, which gave the highest yield.

5.4- Effect of organic matter & ash:

Chen, *et al*. (2000) reported that organic matter contents of the substrates ranged from 55.0 to 62.8% (based on the assumption that carbon constitutes 58% of the OM). It increased after casing and decreased to about 55% during cropping. Our results were in agreement with those of Chen et al. (2000). Organic matter ranged from 50% up to 69% and it was decreased with time because of degradation by microorganisms. It was recorded that O M reduced 30% during crop cycle (Van Griensven, 1988). The lowest O.M (50%) was in 4^{th} flush in room number 2, while the highest O M (69%) was in phase II composting in room number 3. The ash content ranged from 31% up to 50% and it was constant or slightly increased with time. The lowest ash content (31%) was in phase II in room number 3, while the highest content (50%) was in 4^{th} flush in room number 2.

5.5- Effect of ammonia content:

Ammonia was released from the compost during phase I composting, since it reached the highest concentration (full mark on the ammonia measurement), then it decreased to the lowest level (≤ 0.05 %.) through conditioning process at the end of phase II composting when the concentration of ammonia still high (≥ 0.1 %).

In this experiment, the concentration of ammonia was ranged from 0.021 up to 0.10 %. Moreover, it was measured at the end of phase II composting.

The lowest ammonia content(0.0216), was in room number 3 which achieved the highest yield and productivity, while the highest concentration of ammonia (0.1998), was in room number 6 which produced nothing(yield equal zero). Ammonia concentration more than 0.05% was lethal for mushroom mycelium. So the Ammonia

concentration in the compost was the limiting factor for mushroom production. These results were in agreement with that of Rinker (1986) who said that during pasteurization period, the concentration of ammonia was in its high rate, while in the conditioning phase, the concentration decreased gradually until it reached the lowest degree (less than 0.1%).

5.6- Effect of C / N ratio:

Carbon to Nitrogen (C / N) ratio ranged from 10 to 25 during all stages of growth, it was noticed that the C / N ratio decreased during the mycelial growth. The lowest C/N ratio (10) was in phase II composting in room number 6, while the highest C/N ratio was in phase I in room number 4. These results were in agreement with Tajbakhsh, *et al.,* (2008) who reported that total organic carbon (TOC) decreased by 36% by the end of the study period. Also C/ N ratio decreased significantly with time due to substrate decomposition (Tajbakhsh, *et al*., 2008).

5.7- Effect of fungal contamination:

It was noticed that fungal contamination before phase II composting was not important because pasteurization during this phase will kill anything efficiently, and made the compost clean from any contamination. Also it was noticed that at the end of phase II composting there were no fungal contaminants, which means the pasteurization was very effective to eliminate any microorganisms from the compost. However, the fruiting of mushroom depended on the presence of certain organisms, such as; bacteria, Actinomycetes and fungi which are primary decomposers, which secreted exotic enzymes to degrade organic material and converted it to suitable food for the mushroom mycelium, and this was with agreement with that of Fan *et al*., 2000.

The air-borne fungi such as; *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp. and *Cladosporium* sp. were not important because they were found in the environment yet they did not affect the yield of mushroom significantly. The other contaminants such as; *Trichoderma* sp., *Fusarium* sp., *Mycogone* sp., and *Rhizoctonia* sp. were introduced to the growing room only by two ways; spawning and casing, and they spread in the room by workers, irrigation water and instruments. Several species of *Trichoderma* caused green mold disease in edible mushrooms resulting in serious problems for mushroom farmers (Miyazaki, *et al*., 2009).

At any phase of growth an undesirable growth or development of certain molds can occur and can adversely affect the final mushroom yield. The production of fruiting bodies was severely affected by fungal pathogens (Potocnik, *et al*., 2008).

Bacterial and fungal diseases were major problems in mushroom cultivation; a high percentage of products was lost due to lower productivity, decrease of quality and shortened shelf life. The white button mushroom *Agaricus bisporus* was highly sensitive to microbial diseases (Sokovic and Van Griensven, 2006).

Different contaminants were associated with different stages of mushroom cultivation. The disease severity and symptoms depended on the stage of mushroom development at the time of infection (Potocnik, *et al*., 2008). In compost culture, the major contributors to contamination were the materials used, the spawn, the workers or the facilities. These organisms were encouraged during the preparation of compost or during spawn run. *Rhizopus* sp. appeared in rooms numbers 1, 2, 3, 4, and 5 during different growth stages; fourth flush (rooms 1 & 4), casing stage (rooms 2 & 5), phase I composting (room number 3) with number of CFUs of 62.5, 51, 171, 131.5, and 30, respectively. It had high density during phase I composting and during fourth flush (spent compost). *Penicillium* sp. appeared only in two rooms, rooms number 1 and 4 (both during fourth

flush), with number of CFUs of 50 and 27. *Cladosporium* sp. appeared only in room number two (during fourth flush), with number of CFUs of 175, but it was with no importance because it was appeared too late in the season. *Aspergillus* sp. appeared early in room number 4 (during spawning stage), with number of CFUs of 22, but it appeared too late in room number 3 (during fourth flush), with number of CFUs of 25. This fungus was with no importance because it did not cause any significant losses to mushroom yield during any stage of infection. *Trichoderma* sp. was appeared in rooms number 3, 4 and 5 (during casing stage, spawning stage, and phase I composting), with numbers of CFUs of 300, 84, 300, and 54). It was suggested that the appearance of this fungus during two stages in the same room (phase I composting and during spawning in room number 5), that the pasteurization process was not correct and it did no kill all the populations of the fungus, and this infection affected the yield negatively (4400kg). *Mycogone* sp. appeared only in one room number 2 (during third flush) with number of CFUs of 800.3, it was heavily infected the compost, and caused a disease called wet bubble, but the effect on the yield was not obvious because the infection occurred lately during the end of third flush. *Fusarium* sp. also appeared only in one room, number 4 (during the third flush), with number of CFUs of 50, but despite the moderate infection, the effect on yield of mushroom was very high and the loss of production was about 50% (2465.6kg only). The most important fungus was *Rhizoctonia* sp. appeared early during spawning in room number six, with number of CFUs of 320, this fungus appeared as a powdery white mold which colonize the compost and caused apportion of primordial formation which prevented pin head appearance, and there were no yield as a result of this infection. This fungus was not mentioned in mushroom diseases, so there was no information documented about it.

This result was in agreement with that of Vajna *et al*., (2010), who said that: "in the initial phase, different *Penicillium, Fusarium, Aspergillus, Cladosporium,* and *Trichoderma* strains were detected most frequently, in addition to *Alternaria* and *Mucor* species. After pasteurization, no fungi could be isolated with the applied methods (Vajna, *et al*., 2010).

It was able to distinguish between so-called competitive molds and parasitic molds, competitive molds were molds which adversely affect the growth of the mushroom mycelium, during the spawn run or colonization of the substrate (compost and/ or casing layer) through competition for CO_2, nutrients, water and space. Although parasitic molds can also exhibit this competitive behavior to greater or lesser extent, they are different from the competitive category of moulds in that they damage the mycelium and/or the fruit bodies of the mushroom. Competitive moulds were also described as weed moulds to indicate that they are undesirable and were spread quickly and on a massive scale, or they were also known as indicator moulds. When harmful fungi appeared in the compost during growing, it was no longer being possible to control them. Slight infections of fungi on the casing peat moss during growing was able to be controlled to some extent with certain pesticides. So a rapid detection method for the identification of *T. harzianum* was important for decreasing damage in mushroom cultivation (Miyazaki, *et al*., 2009).

5.8. Yield & productivity:

The highest yield (5744.1 kg) was in room No. 3, then room number one came in the second stage and room number two in the third class with quantity of yield equal 5533.8 kg and 5140.7 kg, respectively. In the other hand, room number six was the lowest in production (zero kg), while room number five produced more than room number four,

with quantities equal 4400 kg and 2465.6 kg, respectively. Also productivity was in the same style because the growing area and quantity of compost for all the rooms were the same. In room number three which produced the highest yield all physical and chemical analysis were normal, pH ranged from 6.55 in the first flush up to 7.10 in phase I composting. Bulk density increased gradually from 0.58 in phase I composting to 0.72 in the fourth flush, while porosity decreased gradually from 96% in phase I and phase II composting to 87% during the fourth flush. Moisture content was approximately constant about 60% in all stages, with some exceptions in phase I composting it was 65% and in third flush it was decreased to 50%. In the same manner, dry matter was constant about 40% except in phase I composting it decreased to 35% and in the third flush it was raised to 50%. Also ash content fluctuated from 31% during phase II composting to 47% during flush four. Carbon / nitrogen ratio ranged between 12.5 in flush four and 16 during spawning, casing and second flush. Ammonia concentration was the lowest in all rooms (0.02160 %) and it was believed that ammonia concentration was the limiting factor for mushroom quantity produced and productivity. Finally, fungal contamination in room number three was *Rhizopus stolonifer* in phase I composting, *Trichoderma harzianum* in casing, and *Aspergillus niger* during fourth flush, which was with no effects on yield and productivity.

According to the fungal contamination in room number four, it was infected with *A. niger* and *T. harzianum* during spawning and also infected with *Fusarium* sp. during the third flush which affected the yield negatively and decreased the production of third and fourth flushes to 320.1 kg and 512 kg, respectively, which caused a significant reduction in total yield. The room was infected with *R. stolonifer* during fourth flush.

The lowest yield and productivity were in room number four which were 2465.6 kg and 12.3 respectively. In this room, the pH value was ranged between 6.54 during third flush and 7.2 during phase II composting. Bulk density increased from 0.62 during phase I composting up to 0.78 during fourth flush, while porosity decreased from 95% during phase I, and phase II composting to 89% during first, second and fourth flushes. Moisture content decreased from 72% during phase I composting to 60% during casing, first and third flushes, while dry matter increased from 28% during phase I composting up to 40% during casing, first and third flushes. Organic matter decreased from 68% during phase II composting to 53% during fourth flush, while ash content increased from 32% during phase II composting up to 47% during fourth flush.

Carbon to Nitrogen (C/N) ratio decreased from 22.8 during phase I composting to 11 during first and fourth flushes.

In room number six which produced nothing (no yield), the infection with *Rhizoctonia* sp. (powdery white mold fungus) which appeared early in spawning stage and grown quickly and spread all over the boxes and covered the compost and casing layer in few days. Spraying with fungicides such as Rizolex and Benomyl 50% on the surface of infected boxes was with no effect in controlling the disease. The fungus was very aggressive and competed vigorously with the mushroom mycelium, which retarded the growth of mycelium and caused primordial malformation, and the result was no fruiting bodies were formed and the yield was zero. Organic matter was ranged from 53.9 to 56.6, while ash content ranged from 43.4 to 46.1, and C/N ratio ranged between 9.9 and 12.4. The ammonia concentration was the limiting factor and the clear evidence that the compost was not with good quality for mushroom production; it was the highest concentration in all the growing rooms (0.19980 %). The growth of mycelium was determined by calculating the days between spawning and the first flush, which was

represented as earliness. It was ranged between 36 days (in rooms 1, 2, 3) and 39 days in room number 5. The scheme of flushes was ranged between 7 – 14 days. The normal period between flushes were 7 days (as in second flush in room number 2, second flush in room number 3, third and fourth flushes in room number 5), but sometimes the flush was delayed as a result of market demand, so the grower forced to delay picking of mushroom to allow more fruits to mature, and increase the yield. Our results were in agreement with that of Miller, *et al*., (1990) who produced average fresh mushroom yields of 19.4 kg per bed m^2 (range 17.3 – 22.7 kg / m^2), or 0.66 kg per kg dry compost, in a 3 flush- cropping cycles(Miller, *et al*., 1990), with more expanded range (14.6 – 34.2 kg / m^2).

5.9. Dry matter of fruiting bodies of mushrooms in different flushes.

Dry matter of fruiting bodies of both caps and stems of mushrooms during four flushes was determined (Table: 9). It was ranged between 8.06 and 9.6, and there were no significant differences between different flushes, and also there was no significant difference between caps and stems. Colak, *et al*., (2007) studied dry matter of *A. bisporus*, and they found that dry matter values ranged from 7.74 to 11.46%. Our results were in agreement with Chang, 1999, who said that " the moisture content of fresh mushrooms varies within the range 70 – 95% depending upon the harvest time and environmental conditions (Chang, 1999). About 7 – 10% of the mushroom consists of dry matter (Gerrits, 1988). Our results also agreed with those reported by Colak *et al*., 2007 and with Gerrits, 1988.

5.10. Brown strain (Portobello):

Brown strains are cultivated only on small scale in many countries, such as West Germany and Switzerland. They are highly resistant to diseases and they fetch a good price. The color ranges from the dark brown to the very light brown depending on the strain. The stem remains white, and the wild strain of *A. bisporus* is brown (Anderson,

*et a*l., 2001). Our results in Jordan showed that brown strains did not favored in the market and their price was less than white mushrooms. Its advantages were the tolerant for stress and diseases, more than white strains, earliness in maturity and bigger size when mature (cap diameter ranged from 60 cm – 90 cm).

6. Conclusions:

1) By recycling agricultural wastes instead of burning them, raw materials can actually be used for the cultivation of mushroom.

2) Growers must take in consideration the effect of raising pH value more than 8 during crop cycle.

3) Bulk density of the compost can affect yield and productivity of mushroom negatively when it increased more than 700kg / m^3, while porosity can affect them negatively when it decreased less than 88%.

4) Compost organic matter between 52% - 70% can achieve high yield and productivity, while ash content between 30% - 45% can give the same result.

5) The most important factor which must be noticed is the concentration of ammonia and its negative effect on mycelial growth of mushroom when it raise above 0.05 % in the growing rooms.

6) Carbon / nitrogen ratio of the compost must be in the range of 12 – 25, otherwise it will affect yield and productivity negatively.

7) Microbial contamination must be noticed during the crop cycle of mushroom growth, especially certain fungi as, *Fusarium* and *Rhizoctonia* which affect negatively production and productivity of mushroom.

7. Recommendations:

1) Studies must be conducted to evaluate pasteurization process on different temperatures and their effect on compost properties.

2) Studies must be conducted on other agro wastes as compost ingredients which are more available in Jordan, and cheaper than wheat straw.

3) Studies must be conducted on other species of mushroom which are more tolerant and less susceptible than *Agaricus sp*. to environmental conditions and diseases.

4) Studies must be conducted to evaluate the effect of other physical and chemical properties of compost such as ventilation, light and minerals on yield and productivity of mushrooms.

5) Studies must be conducted to evaluate the effect of other microbial contaminants on yield and productivity of mushrooms.

6) Spent compost can be used as soil fertilizer which can provide the plants with plenty of minerals.

7) Growers in Jordan can establish a compost factory to manufacture their requirements of compost in a scientific manner.

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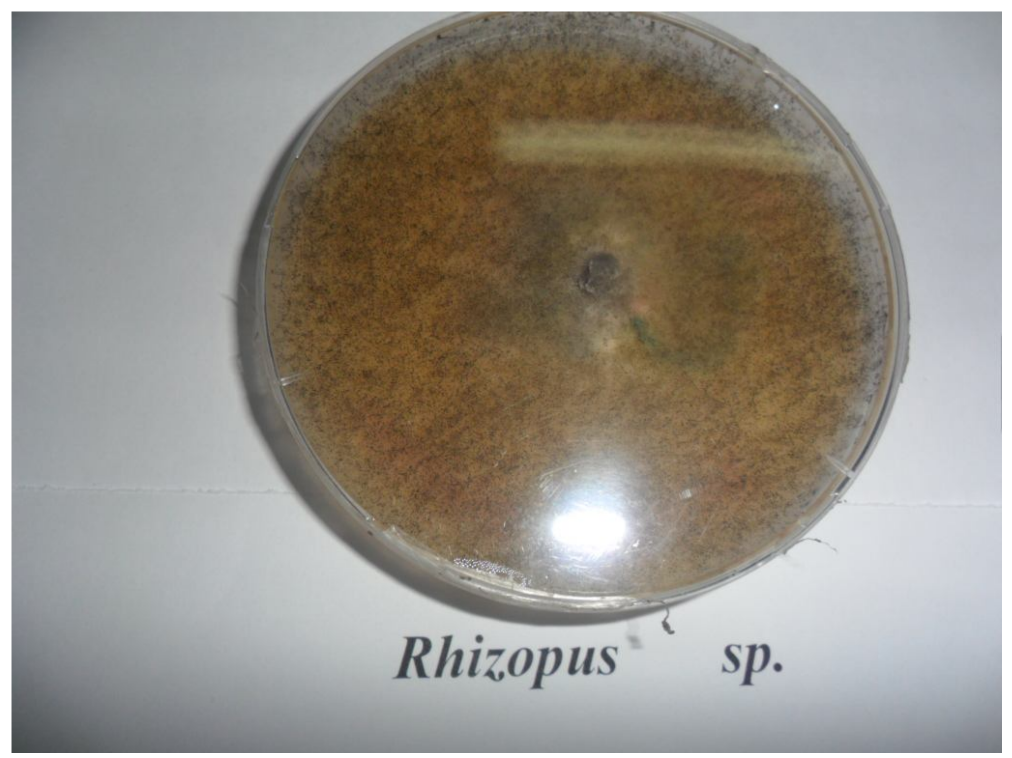
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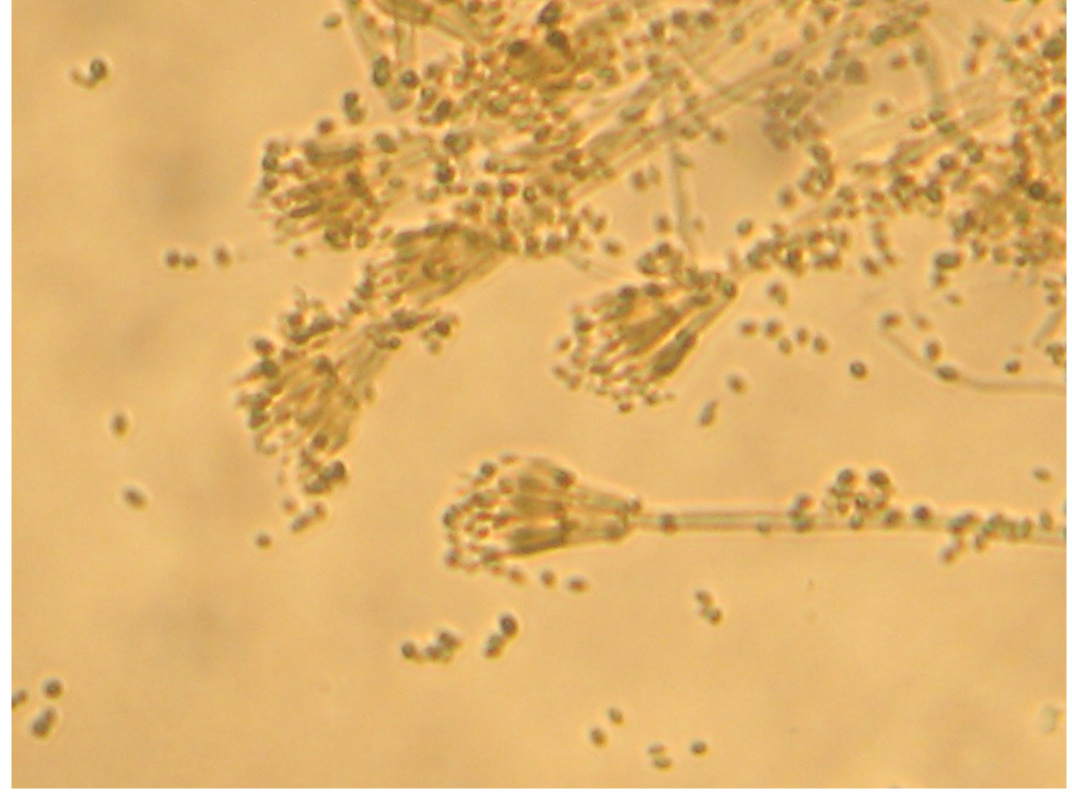
(Figure: 1): Growth of *Rhizopus stolonifer* on potato dextrose agar (PDA)



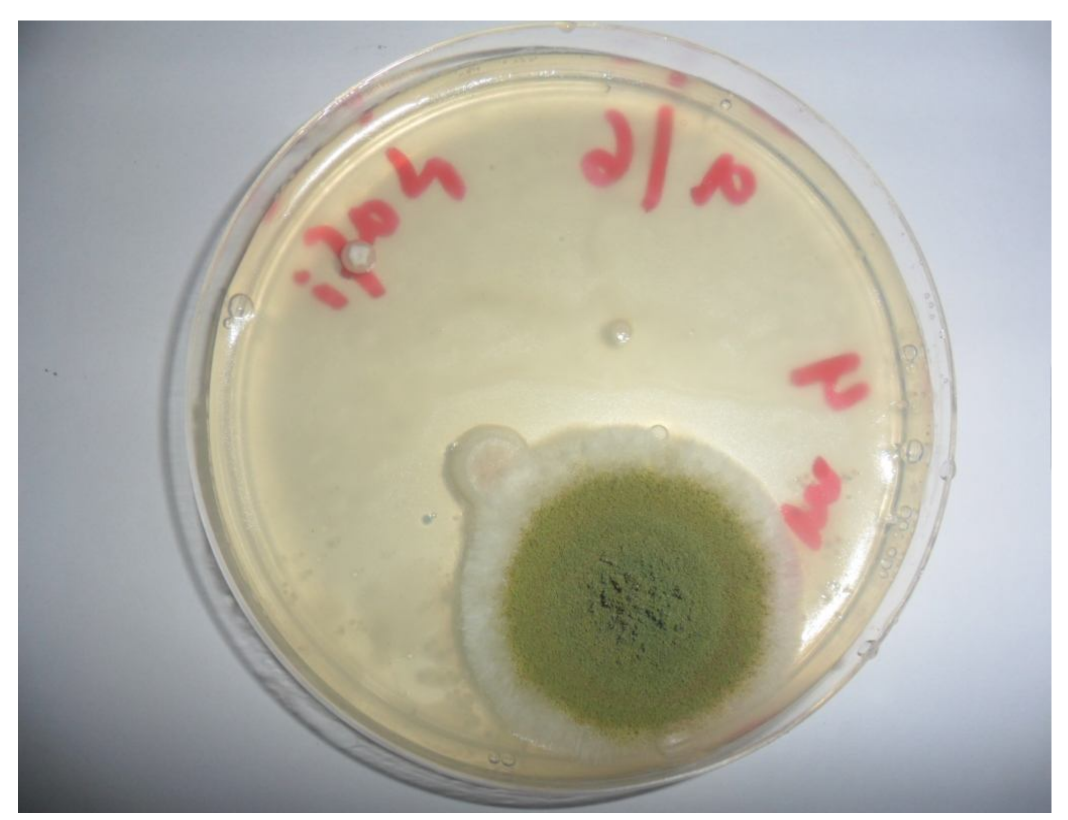
(figure: 2) *Rhizopus stolonifer* (Magnification 400x)



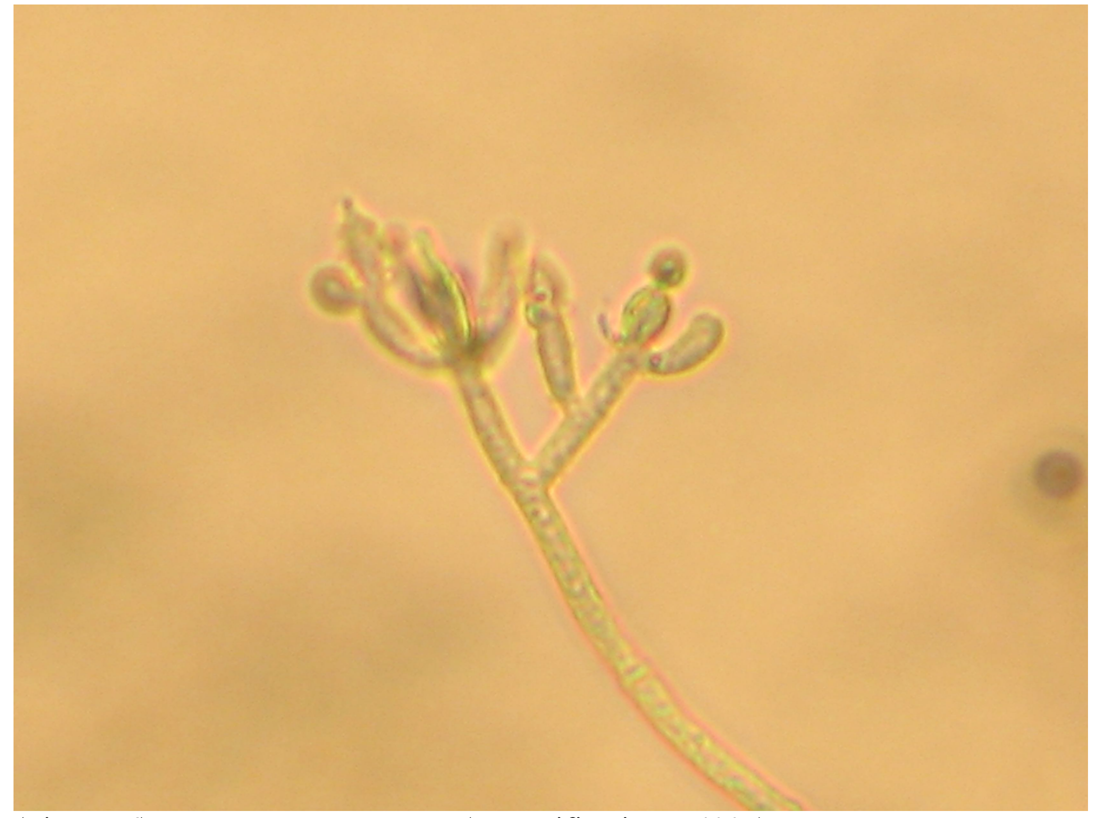
(Figure: 3): Growth of *Penicillium digitatum* on potato dextrose agar (PDA).



(Figure: 4) *Penicillium digitatum* (magnification = 400x).



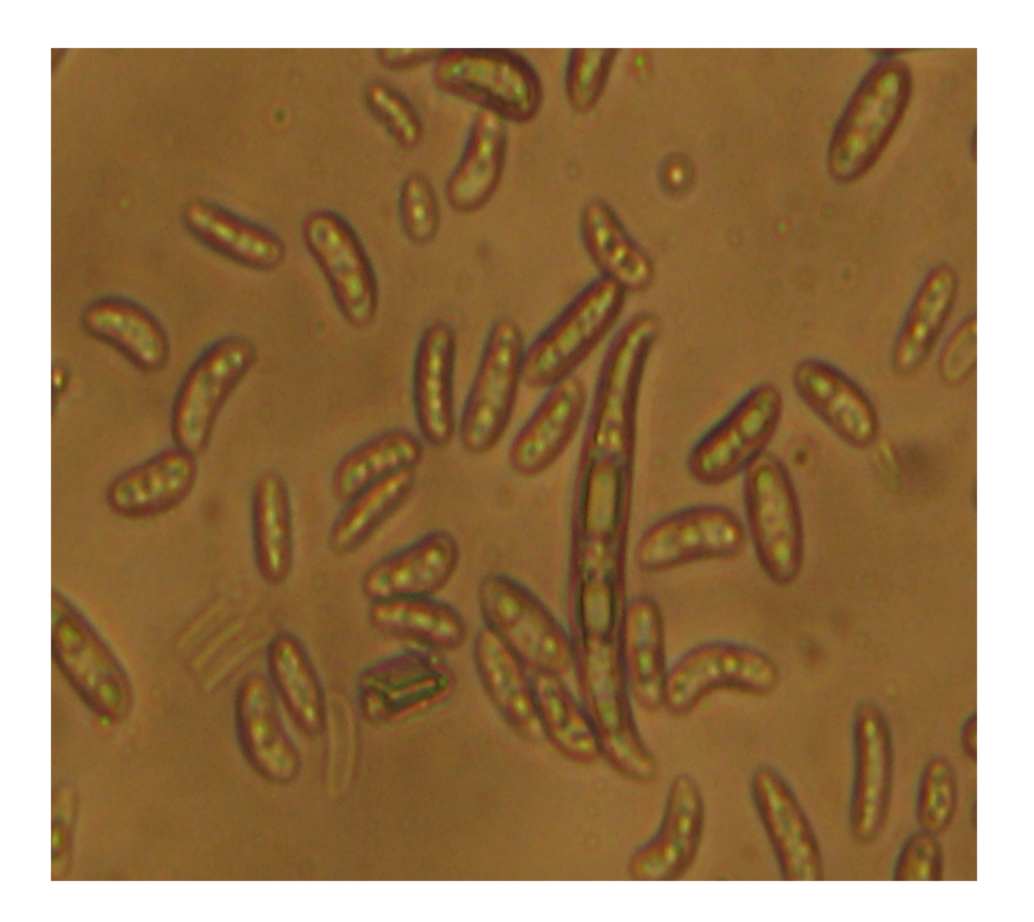
(Figure: 5): Growth of *Mycogone perniciosa* on potato dextrose agar (PDA).



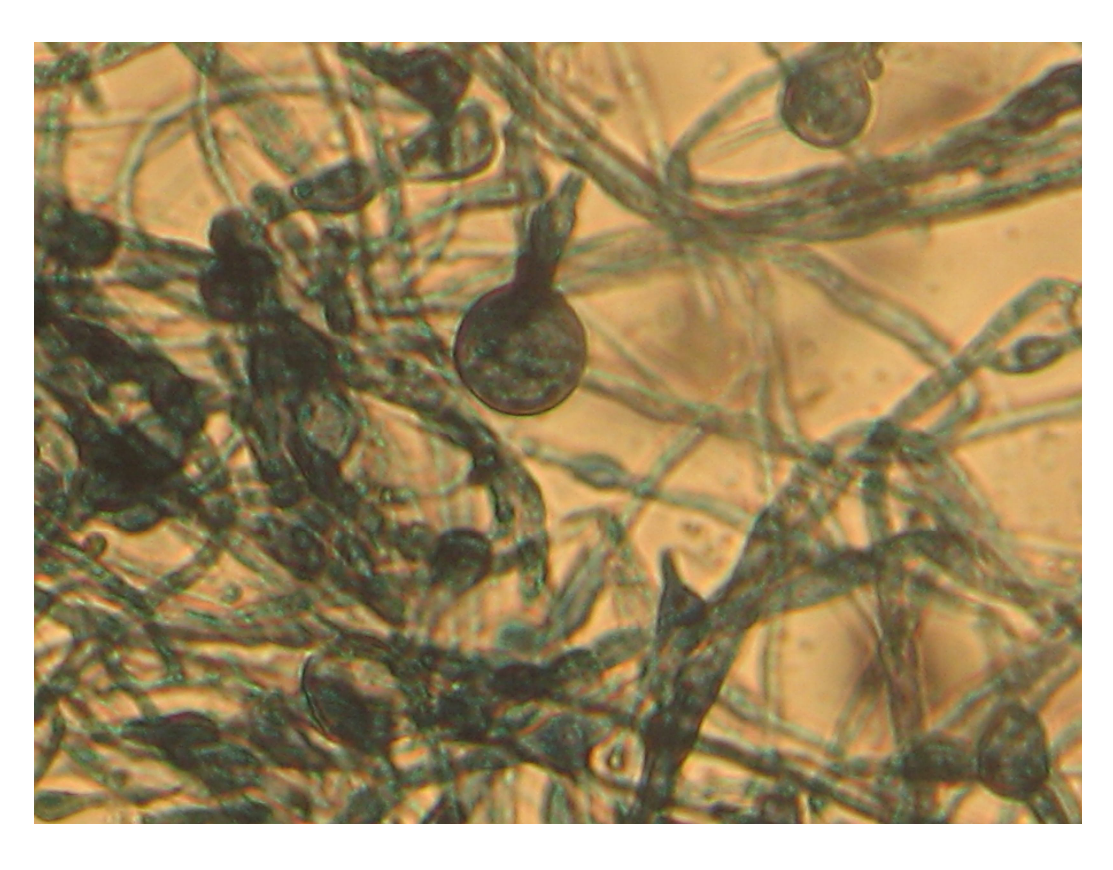
(Figure: 6): *Mycogone perniciosa.* (Magnification = 400x).



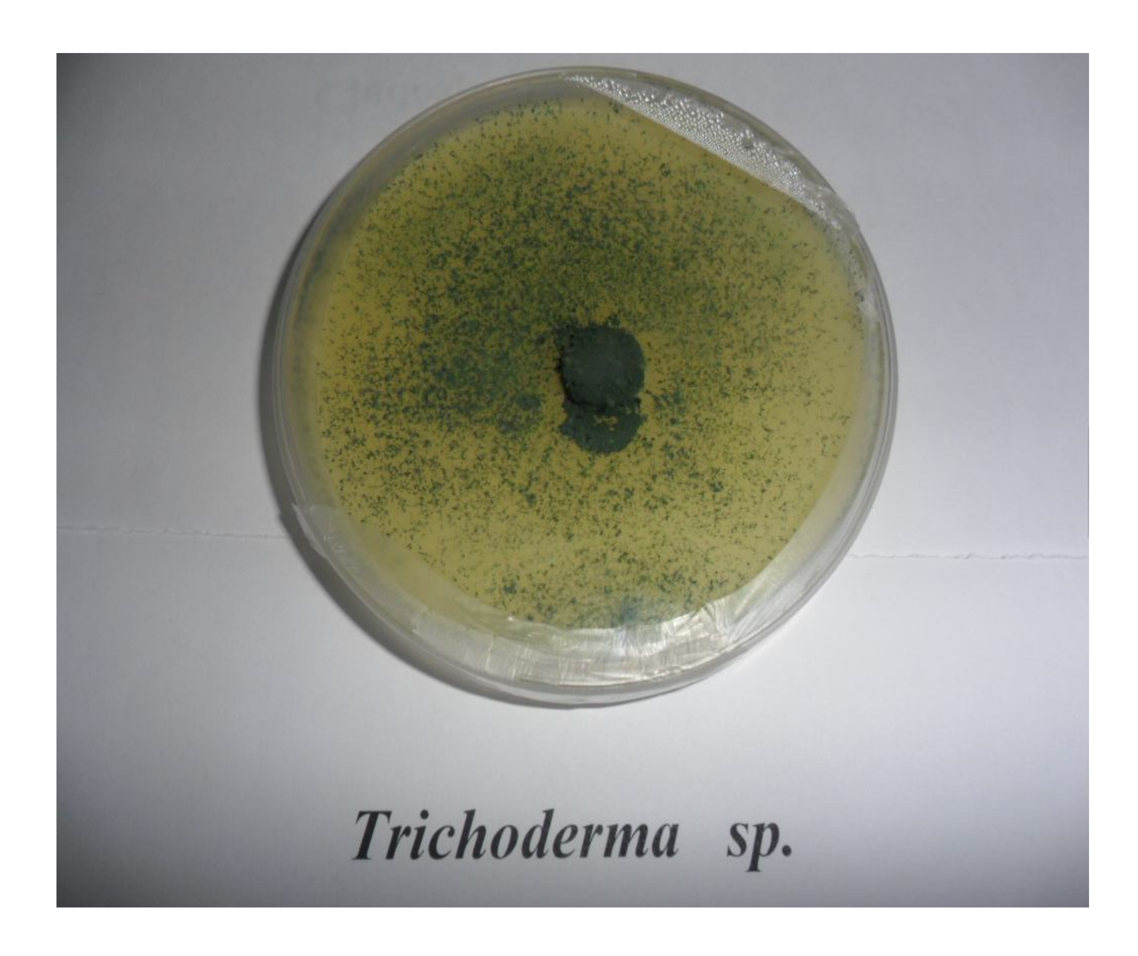
(Figure: 7): Growth of *Fusarium moniliforme* on potato dextrose agar (PDA) medium.



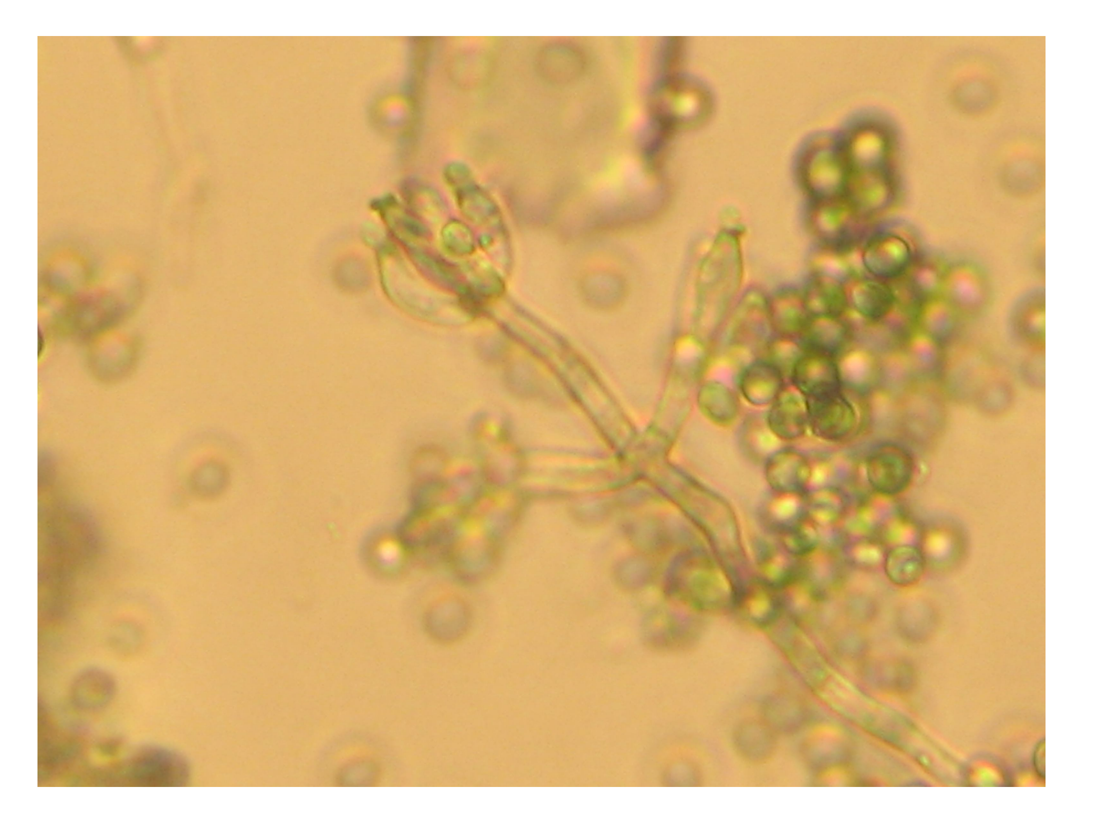
(Figure: 8): *Fusarium moniliforme* (Micro + macro conidia)(400x).



(Figure: 9): Clamydospores of *Fusarium moniliforme*(400 x).



(Figure: 10): Growth of *Trichoderma harzianum* on potato dextrose agar (PDA).



(Figure: 11) *Trichoderma harzianum* (Magnification = 400x).



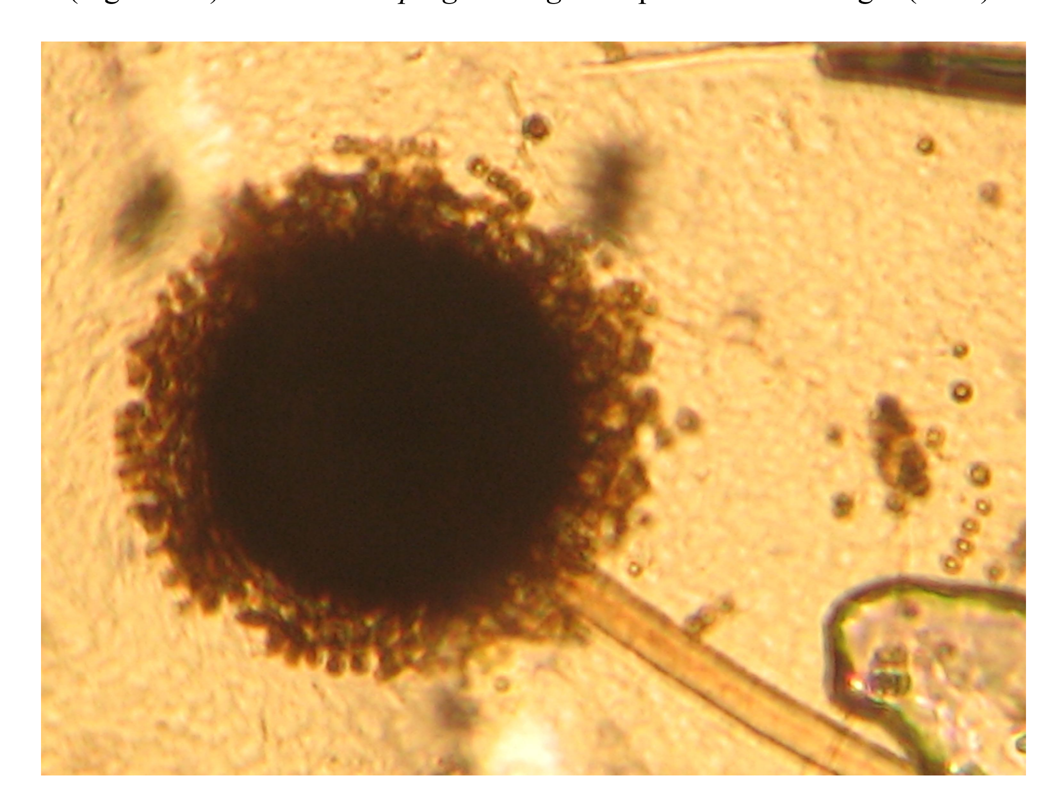
(Figure: 12) Growth of *Cladosporium herbarum* on potato dextrose agar (PDA).



(Figure:13) *Cladosporium herbarum* (magnification = 400x).



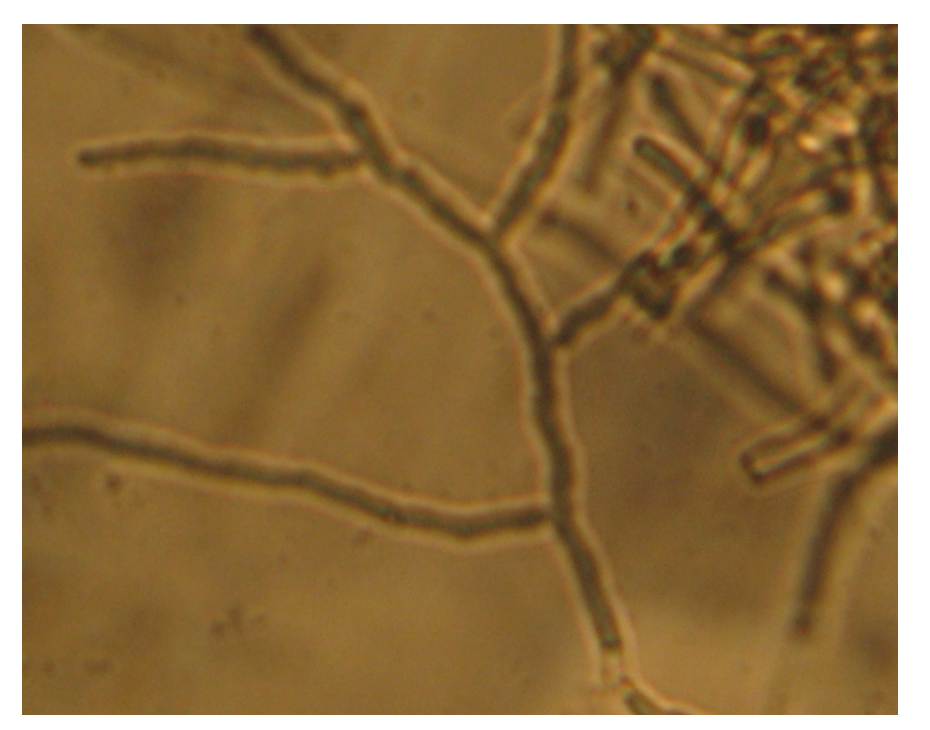
(Figure :14) Growth of *Aspergillus niger* on potato dextrose agar (PDA).



(Figure: 15) *Aspergillus niger* (Magnification = 400x).



(Figure: 16): Growth of *Rhizoctonia solani* on potato dextrose agar (PDA).



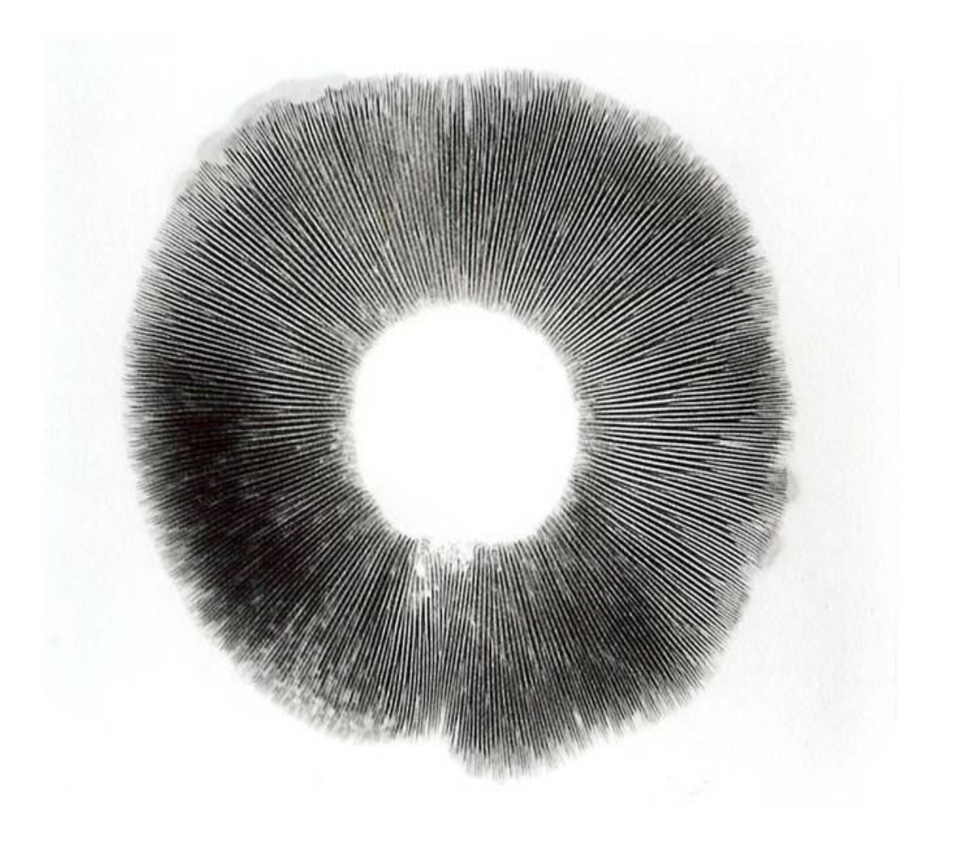
(Figure: 17) *Rhizoctonia solani* (magnification = 400x)



(Figure: 18): wheat straw



(Figure: 19) Growth of *Agaricus bisporus* on potato dextrose agar PDA.



(Figure: 20) spore print of *A. bisporus*.



(Figure: 21) Mature fruits of white strain of *A. bisporus*.



(Figure: 22) growing room (stainless steel).



(Figure: 23) New compost in growing room.



(Figure: 24) Mushroom yield of white strain of *A.bisporus.*



(Figure: 25) Portobello mushroom

Appendices

Appendix 1: physico- chemical properties of wheat straw (main ingredient of compost)

Sample	pH	Moisture %	Dry matter %	Bulk density (g/ml)	Organic matter %	Ash %	T O C %	N%	C/N Ratio
Sample 1	6.4	9.4	90.6	0.2	91.5	8.5	49.3	0.87	56.6
Sample 2	6.4	8.7	91.3	0.2	92.9	7.1	48.2	0.74	65.1
Sample 3	6.4	7.3	92.7	0.2	91.8	8.2	49.4	0.78	63.3
Average	6.4	8.5	91.5	0.2	92	8	49.5	0.8	61.9

Appendix 2: physico- chemical properties of broiler chicken manure (main nitrogen source in compost).

Sample	pH	Moisture %	Dry matter %	Bulk density (g/ml)	Organic matter %	Ash %	T O C %	N %	C/N Ratio
Sample 1	7.40	5.2	94.8	0.45	78.7	21.3	42.6	4.4	9.7
Sample 2	7.32	5.5	94.5	0.47	79.6	20.4	43.1	4.8	8.9
Sample 3	7.35	5.3	94.7	0.47	79.7	20.3	43.1	4.7	9.2
Average	7.35	5.3	94.6	0.46	79.3	20.6	42.9	4.6	9.3

Appendix 3: physico- chemical properties of compost in growing room number 1.

Room No. 1: Ammonia concentration at the end of phase II composting = 0.0225 %.

Date	Stage	pH	Porosity %	Bulk density (g/ml)	Moisture %	Dry matter%	Organic matter%	Ash%	TOC %	N%	C : N ratio	Production (Kg)
09/07	Phase I	7.20	96	0.65	69	31	58.4	41.6	32.0	2.4	13.3	
30/07	Phase II	7.18	95	0.66	62	38	58	42	31.86	2.45	13.0	
07/08	Spawning	7.16	95	0.66	60	40	57.8	42.20	31.80	2.50	14.0	
21/08	Casing	6.80	93	0.70	60	40	55.8	44.2	30.7	1.96	12.7	
12/09	1st flush	6.68	92	0.72	58	42	57.5	42.5	31.6	2.56	12.3	1563.4
20/09	2nd flush	6.55	91	0.75	60	40	59.0	41.0	32.38	1.99	16.3	1249.5
30/09	3rd flush	6.66	90	0.76	55	45	55.4	44.50	30.54	2.08	14.7	950.9
14/10	4th flush	6.57	88	0.78	60	40	52.7	47.30	29.10	2.34	12.4	1770
	Total											5533.8

Appendix 4: physico- chemical properties of compost in growing room number 2.

Room No. 2: Ammonia concentration at the end of phase II composting = 0.02558 %.

Date	Stage	pH	Porosity %	Bulk density (g/ml)	Moisture %	Dry matter %	Organic matter %	Ash%	TOC %	N%	C : N ratio	Production (Kg)
20/09	Phase I	7.75	96	0.60	68	32	59	41	32.38	2.82	11.48	
10/10	Phase II	7.53	95	0.64	66	34	58	42	31.86	2.26	14.09	
17/10	Spawning	6.94	94	0.66	62.5	37.5	56	44	30.82	2.38	12.95	
31/10	Casing	6.67	93	0.68	57	43	57.80	42.20	31.76	2.44	13.0	
22/11	1st flush	6.65	92	0.71	53.8	46.2	56.2	43.8	30.92	2.50	12.37	976.3
29/11	2nd flush	6.58	91	0.72	51.8	48.2	55.8	44.2	30.72	2.56	12	940.5
10/12	3rd flush	6.66	91	0.72	55	45	55.45	44.55	30.54	2.08	14.68	800.3
20/12	4th flush	6.62	89	0.75	60	40	50.30	49.70	27.80	2.61	10.65	2423.6
	Total											5140.7

Appendix 5: physico- chemical properties of compost in growing room number 3.

Room No. 3: Ammonia concentration at the end of phase II composting = 0.0216 %.

Date	Stage	pH	Porosity %	Bulk density (g/ml)	Moisture %	Dry matter %	Organic matter %	Ash %	TOC %	N%	C : N ratio	Production (Kg)
01/11	Phase I	7.1	96	0.58	65	35	65.8	34.20	35.9	2.52	14.25	
21/11	Phase II	7.0	96	0.60	60	40	69.5	30.50	37.8	1.40	24.82	
28/11	Spawning	6.97	96	0.65	60	40	57	43.00	32	1.75	18.28	
12/12	Casing	6.97	94	0.66	60	40	58.41	41.59	32.07	1.96	16.4	
03/01	1st flush	6.68	93	0.68	60	40	59.20	40.78	32.5	2.52	12.8	1585
10/01	2nd flush	6.55	93	0.69	60	40	59	41.00	32.38	1.99	16.27	1395.3
20/01	3rd flush	6.66	92	0.69	58	42	55.45	44.50	30.54	2.08	14.68	1033
30/01	4th flush	6.57	91	0.72	60	40	52.70	47.30	29.10	2.34	12.4	1730.8
	Total											5744.1

Appendix 6: physico- chemical properties of compost in growing room number 4.

Room No. 4: Ammonia concentration at the end of phase II composting = 0.09816 %.

Date	Stage	pH	Porosity %	Bulk density (g/ml)	Moisture %	Dry matter %	Organic matter %	Ash%	TOC %	N%	C : N ratio	Production (Kg)
01/12	Phase I	7.1	96	0.62	72	28	67	33	36.5	1.6	22.8	
22/12	Phase II	7.2	95	0.65	68	32	68	42	37	1.8	20.5	
30/12	Spawning	7.16	93	0.70	66	34	58	42	31.4	2.6	12.5	
15/01	Casing	6.89	91	0.72	60	40	58	42	31.8	2.5	13	
06/02	1st flush	6.78	89	0.75	60	40	57	43	31.3	2.9	11	682.6
16/02	2nd flush	6.66	89	0.77	65	35	56	44	30.8	2.43	12.5	950.9
26/02	3rd flush	6.54	90	0.75	60	40	55	45	30.3	2.54	12	320.1
12/03	4th flush	6.55	89	0.78	62	38	53	47	29.3	2.62	11	512
	Total											2465.6

Appendix 7: physico- chemical properties of compost in growing room number 5.
Room No. 5: Ammonia concentration at the end of phase II composting = 0.0861 %.

Date	Stage	pH	Porosity %	Bulk density (g/ml)	Moisture %	Dry matter %	Organic matter %	Ash%	TOC %	N%	C : N ratio	Production (Kg)
25/09	Phase I	7.26	94	0.66	66	34	58	42	31.86	2.8	11.4	
16/10	Phase II	7.40	94	0.67	67	33	59	41	32.38	2.55	12.7	
26/10	Spawning	7.74	93	0.68	63	37	59	41	30.15	2.27	13.28	
12/11	Casing	7.20	92	0.70	62	38	58	42	31.86	2.22	14.35	
04/12	1st flush	6.52	91	0.72	61	39	57	43	31.34	2.35	13.34	729
12/12	2nd flush	6.55	89	0.75	60	40	59	41	32.38	2.28	14.20	895.5
19/12	3rd flush	6.60	93	0.71	60	40	56	44	30.82	2.25	13.70	1245.5
26/12	4th flush	6.57	91	0.72	60	40	53	47	29.10	2.34	12.40	1530
	Total											4400

Appendix 8: physico- chemical properties of compost in growing room number 6.

Room No. 6: Ammonia concentration at the end of phase II composting = 0.1998 %.

Date	Stage	pH	Porosity %	Bulk density (g/ml)	Moisture %	Dry matter %	Organic matter %	Ash %	TOC %	N %	C:N ratio	Production (Kg)
15/01	Phase I	8.07	95	0.64	50	50	56.6	43.4	31	2.5	12.4	
10/02	Phase II	7.94	94	0.71	50	50	53.9	46.1	29.7	2.7	9.9	
19/02	Spawning	8.10	92	0.72	50	50	54.6	45.4	30	2.8	10.7	
08/03	Casing	8.04	88	0.77	50	50	55	45.0	30	2.8	10.7	
	1st flush											00.0
	2nd flush											00.0
	3rd flush											00.0
	4th flush											00.0
	Total											00.0

Appendix 9: Effect of fungal contaminant on mycelial growth and yield of *Agaricus bisporus* in growing room No. 1.

Stage	Date	Fungi	CFU	Total Production Kg	Average fruit wt. Grams	Uniformity	Earliness Days	Productivity Kg/ton
Phase I	09/07							
Phase II	30/07							
Spawning	07/08							
Casing	21/08							
1st flush	12/09			1563.4	18	N	36	
2nd flush	20/09			1249.5	22	M	8	
3rd flush	30/09			950.9	24	M	10	
4th flush	14/09	*Rhizopus sp.* *Penicillium sp.*	62.5 50	1770	17	N	14	
Total				5533.8				27.7

CFU: colony forming units / ml = $\frac{\text{No. of colonies}}{\text{Dry weight of sample}} \times \frac{1}{\text{dilution}}$

Appendix 10: Effect of fungal contaminant on mycelial growth and yield of *A. bisporus* in growing room No. 2.

Stage	Date	Fungi	CFU	Total Production Kg	Average fruit wt. Grams	Uniformity	Earliness	Productivity Kg/ton
Phase I	20/09	-						
Phase II	10/10	-						
Spawning	17/10	-						
Casing	31/10	*Rhizopus sp.*	51					
1st flush	22/11	-		976.3	24	M	36	
2nd flush	29/11	-		940.5	25	M	7	
3rd flush	10/12	*Mycogone sp.*	44.4	800.3	27	L	11	
4th flush	20/12	*Cladosporium sp.*	175	2423.6	15	N	10	
Total				5140.7				25.7

CFU: colony forming units/ ml = $\frac{\text{No. of colonies}}{\text{Dry weight of sample}} \times \frac{1}{\text{dilution}}$

Appendix 11: Effect of fungal contaminant on mycelial growth and yield of *A. bisporus* in growing room No. 3.

Stage	Date	Fungi	CFU	Total Production Kg	Average fruit wt. Grams	Uniformity	Earliness Days	Productivity Kg/ton
Phase I	01/11	*Rhizopus sp.*	171					
Phase II	21/11	-						
Spawning	28/11	-						
Casing	12/12	*Trichoderma sp.*	300					
1st flush	03/01	-		1585	18	N	36	
2nd flush	10/01	-		1395.3	20	M	7	
3rd flush	20/01	-		1033	22	M	10	
4th flush	30/01	*Aspergillus sp.*	25	1730.8	17	N	10	
Total				5744.1				28.7

CFU: colony forming units / ml = $\frac{\text{No. of colonies}}{\text{Dry weight of sample}} \times \frac{1}{\text{dilution}}$

Appendix 12: Effect of fungal contaminant on mycelial growth and yield of *A. bisporus* in growing room No. 4.

Stage	Date	Fungi	CFU	Total Production Kg	Average fruit wt. Grams	Uniformity	Earliness Days	Productivity Kg/ton
Phase I	01/12	-						
Phase II	22/12	-						
Spawning	30/12	*Aspergillus sp.* *Trichoderma sp.*	22 84					
Casing	15/01							
1st flush	06/02			682.6	38	L	38	
2nd flush	16/02			950.9	25	M	10	
3rd flush	26/02	*Fusarium sp.*	50	320.1	35	L	10	
4th flush	12/03	*Rhizopus sp.*	131.5	512	30	L	14	
Total				2465.6				12.3

CFU: colony forming units / ml = $\frac{\text{No. of colonies}}{\text{Dry weight of sample}} \times \frac{1}{\text{dilution}}$

Appendix 13: Effect of fungal contaminant on mycelial growth and yield of *A. bisporus* in growing room No.5.

Stage	Date	Fungi	CFU	Total Production Kg	Average fruit wt. Grams	Uniformity	Earliness Days	Productivity Kg/ton
Phase I	25/09	*Trichoderma sp.*	300					
Phase II	16/10							
Spawning	26/10	*Trichoderma sp.*	54					
Casing	12/11	*Rhizopus sp.*	30					
1st flush	04/12			729	27	L	39	
2nd flush	12/12			895.5	25	M	8	
3rd flush	19/12			1245.5	22	M	7	
4th flush	26/12	*Penicilium sp.*	27.5	1530	18	S	7	
Total				4400				22

CFU: colony forming units / ml = $\frac{\text{No. of colonies}}{\text{Dry weight of sample}} \times \frac{1}{\text{dilution}}$

Appendix 14: Effect of fungal contaminant on mycelial growth and yield of *A. bisporus* in growing room No.6.

Stage	Date	Fungi	CFU	Total Production Kg	Average fruit wt.	Uniformity	Earliness	Productivity Kg/ton
Phase I	15/01							
Phase II	10/02							
Spawning	19/02	*Rhizoctonia sp.*	320					
Casing	08/03							
1st flush				00.00	-	-		00.00
2nd flush				00.00	-	-		00.00
3rd flush				00.00	-	-		00.00
4th flush				00.00	-	-		00.00
Total				00.00				00.00

CFU: colony forming units / ml = $\frac{\text{No. of colonies}}{\text{Dry weight of sample}} \times \frac{1}{\text{dilution}}$

تأثير الصفات الفيزيائية والكيميائية والميكروبيولوجية لوسط الزراعة (الكمبوست) على نمو الميسيليوم والانتاج لسلالتين من فطر المشروم "*Agaricus bisporus*".

إعداد
نافع خلف المساعيد

المشرف
الأستاذ الدكتور أحمد محمد الرداد المومني

ملخص

أجريت هذه الدراسة في مزرعة الشركة الرائدة للمشاريع الزراعية – المنارة / الجيزة – طريق المطار (35 كم شرق مدينة عمان) خلال الفترة من حزيران 2010م الى تموز 2011م، على سلالتين من فطر المشروم (*Agaricus bisporus*) والمعروف عالمياً باسم الأزرار البيضاء (White button mushroom)، لدراسة تأثير الصفات الفيزيائية والكيميائية والميكروبيولوجية لوسط الزراعة (الكمبوست) على نمو الميسيليوم والإنتاج لفطر المشروم.

أخذت عينات متجانسة من الكمبوست من غرف الزراعة الستة أثناء مراحل النمو المختلفة، وتم تحليل تلك العينات تحليلاً فيزيائياً وكيميائياً وميكروبيولوجياً لاكتشاف تأثير تلك الصفات على نمو الميسيليوم وكميات الإنتاج للفطر المذكور.

تتم زراعة الكمبوست بأبواغ الفطر بمعدل 600 غرام من البذور لكل 200 كغم من الكمبوست . حيث ينمو الفطر ويستمر في النمو حتى يتنتشر في جميع نواحي الكمبوست، وبعد اسبوعين من الزراعة، يتم تغطية سطح الكمبوست بطبقة رقيقة من البيتموس سمكها 3 – 5 سم مما يؤدي إلى تحفيز الفطر على انتاج اجسام ثمرية (الأزرار). إن عدد وحجم ووزن تلك الأزرار قد تأثر بالصفات الفيزيائية والكيميائية والميكروبيولوجية للكمبوست، فقد تراوحت درجة الحموضة (pH) بين 6.5 و8.1 حيث كانت تتناقص مع مرور الوقت، وقد كانت مؤشراً واضحاً على درجة تجهيز وتعقيم الكمبوست. الكثافة الكلية (bulk density) تراوحت بين 0.58 و 0.78 وقد كانت تزداد مع مرور الوقت، وعلى النقيض من ذلك، المسامية (porosity) التي كانت تتراوح بين 87% و 96%، وقد كانت المسامية تقل بمرور الوقت . إن الكثافة الكلية والمسامية لم يكنا ذات تأثير يذكر على إنتاج الفطر المشروم. لقد تراوح محتوى الرطوبة ما بين 50% و72%، وقد كانت تتناقص مع الوقت،وقد سجلت أعلى مستويات للمحتوى الرطوبي في الجزء الأول من التخمير

في الغرفة الرابعة والتي أنتجت أقل كمية من الفطر المشروم (2465.6كغم)، باستثناء الغرفة السادسة والتي لم تنتج شيئاً، وعلى العكس تماماً كانت المادة الجافة (dry matter) تتراوح بين 28% و 50% والتي كانت تتناسب طردياً مع كمية الانتاج . تراوحت المادة العضوية (organic matter)بين 50% و 69%، ولم يكن للمادة العضوية أي تأثير يذكر على الانتاج، بينما كانت نسبة الرماد(ash) تتراوح ما بين 31% و50%، وكذلك نسبة الرماد لم يكن لها أي تأثير يذكر على كمية الانتاج . تراوحت نسبة الكربون الى النيتروجين (C/N ratio) بين 10 و 22، وقد كانت تتناقص مع مرور الوقت خلال مراحل الانتاج، ولم تكن تلك النسبة ذات أثر مباشر على كميات الانتاج .

تركيز الأمونيا كان هو العامل المحدد الذي يؤثر على نوعية الكمبوست وكان مستوى الأمونيا قد وصل إلى 0.0216% في الغرفة الثالثة والتي أنتجت أكبر كمية انتاج (5744.1 كغم) و 0.1998% في الغرفة السادسة والتي لم تنتج شيئاً بينما في الغرف الرابعة والخامسة والثانية والأولى كان مستوى الأمونيا 0.09816 %،0.0861%،0.02558%،0.0225%، على التوالي،وقد كانت كميات الانتاج والانتاجية على النحو التالي 2465.6 كغم و 12.3،4400 كغم و 22، 5140.7 كغم و 25.7، 5533.8 كغم و 27.7، على التوالي. تم احتساب تركيز الأمونيا بعد انتهاء الجزء الثاني من التخمير، وقد كانت نسبة الأمونيا تتناسب عكسياً مع كمية إنتاج المشروم.

كذلك فقد تمت دراسة أثر التلوث الفطري للكمبوست على نمو الفطر المشروم وانتاجه، فوجد أن بعض أنواع الفطريات كانت خطيرة وأدت الى خسارة كبيرة في الانتاج قد تصل الى 100% خاصة إذا ظهرت الاصابة في وقت مبكر من مراحل الانتاج، حيث كان فطر الرايزوكتونيا *Rhizoctonia* أكثر الفطريات ضراوة في التنافس والتطفل على الفطر المشروم، مما أدى الى خسارة كاملة للمحصول، فقد ظهر الفطر في الغرفة السادسة بعد الزراعة مباشرة، مما أدى الى عدم ظهور الأزرار الحديثة، وبالتالي عدم وجود أي انتاج . فطر الفيوزاريوم (*Fusarium*) ظهر في بداية القطفة الثالثة في الغرفة الرابعة، مما أدى الى خسارة كبيرة في كمية الانتاج للقطفتين الثالثة والرابعة، وهذا بدوره أدى الى خسارة في المحصول الكلي تصل الى 50% من الانتاج (2465.6 كغم) مقارنة مع غيرها من الغرف .

هناك فطريات أخرى منتشرة في الجو ظهرت كملوثات للكمبوست في مراحل مختلفة من النمو مثل: الرايزوبص (*Rhizopus*) و الاسبرجلص (*Aspergillus*) والبينيسيليوم (*Penicillium*) والكلادوسبوريوم (*Cladosporium*) ، ولكن هذه الفطريات لم يكن لها أي أثر يذكر على نمو المشروم أو انتاجه. فطريات أخرى ظهرت أثناء دورة حياة المشروم وفي

غرف مختلفة مثل الترايكوديرما (*Trichoderma*) والمايكوجون (*Mycogone*) ولكن لم يكن لها تأثير يذكر على نمو الفطر وانتاجه. أما إنتاج الفطر المشروم فقد وصل إلى 5744.1 كغم في الغرفة الثالثة و 2465.6 كغم في الغرفة الرابعة (نفس المساحة ونفس الكمية من الكمبوست)، مع استثناء الغرفة السادسة والتي لم تنتج شيئا. الغرفة السادسة لم تنتج شيئاً بسبب إصابتها بفطر الرايزوكتونيا والتي ظهرت في وقت مبكر مباشرة بعد الزراعة،أما الغرفة الرابعة والتي أنتجت أقل كمية من الثمار (2465.6 كغم)، وذلك نتيجة لاصابتها بفطر الفيوزاريوم مع بداية القطفة الثالثة،مما أدى الى نقص كبير في الانتاج (حوالي 50%) . ان الأصناف البيضاء من الفطر المشروم (*Agaricus bisporus*) كانت أقل انتاجية وأكثر شعبية لدى المستهلك في السوق الأردني مقارنة مع الأصناف ذات اللون البني (المسماه بورتوبيللو)، والتي كانت أكثر تحملاً للظروف البيئية غير الملائمة كالحرارة والرطوبة، وأكثر تحملاً للأمراض. أخيراً إن المادة الجافة في ثمار المشروم قد تراوحت بين 8.06 و 9.6 % ولم تكن هنالك فروق معتبرة بين الثمار من قطفات مختلفة، كما لم تكن هنالك فروق معتبرة بين القبعات والسيقان .